ACTA PÆDIATRICA SCANDINAVICA

VOL. 69 / JAN. 1980 / NO. 1

)01-656X

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DISTRIBUTED BY THE ALMQVIST & WIKSELL PERIODICAL COMPANY STOCKHOLM SWEDEN

ACTA PÆDIATRICA SCANDINAVICA

Subscription and Distribution

The Almqvist & Wiksell Periodical Company
26 Gamla Brogatan,
S-101 20 Stockholm 1, Sweden

Printers

Almqvist & Wiksell Tryckeri AB
S 751 81 Uppsala, Sweden

Acta Pædiatrica Scandinavica publishes papers ir lish on scientific investigations in the field of paedi Each issue consists of about 100 pages, six nu forming one volume The numbers will be issued second month *Supplements* will be delivered tc scribers free of charge

Acta Pædiatrica Scandinavica will consider p from authors in all countries Articles are acc for publication on condition that they are contri solely to this journal and have not been pub elsewhere previously Original articles should r scientific findings within the paediatric field of int Review articles should contain an objective ac of different points of view and an internatic representative bibliography Case reports should tain accounts of rare syndromes, new diagnostic and methods, new kinds of treatment and labor research with foreseeable practical application L to the Editor is intended to be a forum for comn on current literature, short discussions and preliminary communications which are thought t important and urgent enough to warrant rapid lication

All papers are subject to editorial revision to r them conform to the style adopted by Acta Pædia Scandinavica A paper should not exceed 6 pri pages, including space for figures and tables Ex tions are occasionally allowed but the author wi charged for the exceeding pages Tables and illu tions covering more than three pages in original art and two pages in Case reports are also printed at author's expense Monographs and more exter articles may be published separately as Suppler at the author's own expense.

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THE ROSÉN VON ROSENSTEIN AWARD

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In 1964 the medal was presented to Professors Guido Fanconi, Zurich, Charles Janeway, Boston, Massachusetts, Arvid Wallgren, Stockholm, and Arvo Ylppo, Helsinki, in 1969 to Professors Josef Houstek, Prague, Derrick Jelliffe Kingston and Alfred Sundal, Bergen, and in 1974 to Professor Samuel Fomon, Iowa City, Iowa, Dr Ronald MacKeith, London, and Professor Bo Vahlquist, Uppsala

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Nils Rosen von Rosenstein and his Textbook on Paediatrics *Acta Paediatr Scand* Suppl 156 1964

Donald Court

You once told me that your career had been purely British and had no international impact I am sure that you meant it, and I am convinced that your statement was a true expression of personal modesty But it was wrong!

Your research in the fields of respiratory infections and the epidemiology of disease in childhood has made your name known and valued far outside Great Britain

Your comprehensive, penetrating and long-continued studies Growing up in Newcastle upon Tyne and The School Years in Newcastle upon Tyne are a landmark In fact they constitute a new and important approach to research in child health and have contributed greatly to the advancement not only of social but also of clinical paediatrics We all know that these investigations were planned and pursued by a team of paediatricians and social scientists but we know too that you were an inspiring leader upon whom the others relied And you can rest assured that these two books have been carefully studied in most parts of the world Although written some time ago, they are still of continuing interest and will certainly remain so far many years to come On this special occasion it seems important to emphasize that these studies of yours apply and extend ideas already perceived by Nils Rosen von Rosenstein Unmistakeably there is a common ground and the same kind of greatness

Furthermore, after you had retired from the James Spence Chair of Child Health in Newcastle, which you held from 1955 to 1972, you still continued your efforts to improve the health and wellbeing of children The two books "The Medical Care of Children" and "Paediatrics in the 70s"—the first expressing the philosophy and practice of a university de-

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partment, the second the considered view of the British Paediatric Association—bear witness to this But, most of all, your chairmanship of the Committee on the Child Health Services and the report "Fit for the Future" which followed, give us reason to express our admiration and gratitude This many-sided report is a real gift to children, parents and paediatricians all over the world To illustrate its value and importance I will quote the words of Professor Otto Wolff when you received the 1978 James Spence Medal "The report Fit for the Future bears Donald's stamp on every page—written in beautiful English, quite unlike the ugly verbiage of many such reports—full of compassion, clearly reasoned, and based on much careful research That its forward looking recommendations have only in part been accepted by the profession and government is a disappointment to many of us, but it is not unexpected that a document of such vision is a decade or so ahead of its time Rosén von Rosenstein was also ahead of his time The first noticeable effects of his work did not appear until half a century after his death, and his textbook on paediatrics was still in use in many European countries a century after its publication

On this occasion, too, it would be an unforgiveable ommission if I failed to mention that you are regarded not only as a scientist of high repute but also as a kind, understanding, and helpful man of the highest moral integrity Personally I shall never forget the warm and serious interest shown to me when long ago, I visited your department in Newcastle It was an extremely stimulating experience for a young and searching colleague from abroad

Stig Sjolin

Acta Pædiatr Scand 69 3–11, 1980

CHILD HEALTH IN A CHANGING SOCIETY

DONALD COURT

I INTRODUCTION

I begin with a tribute to Nils Rosen von Rosenstein, the Father of European Paediatrics, because his approach is still relevant to the work of paediatricians everywhere That is a substantial claim and my task is to justify it in this paper I am encouraged by the fact that Rosen himself lived in a changing society, where scientific enquiry was rising from the ashes of military defeat and economic break down

The convictions of which he would remind us today, might be these 'We must hold firm to an approach based on a fundamental division of patients into children and adults Children are attacked by specific diseases which require specific therapy, and these diseases or deficiency states can be prevented ' One key to advance is measured observation and the preventive use of statistical data Although an outstanding clinician, the late Professor Wallgren considered that Rosen's most important contribution was as a promoter of health and of better understanding among physicians and mothers of the needs of children And in Professor Lind's view the idea of popular enlightenment was not a passing fancy but a consistent feature of his achievements How modern he sounds

II A CHANGING SOCIETY

I shall consider how in Britain we are trying to relate health services for children to their present needs The first step was a stimulus, the stone thrown into the sleeping pool This was provided by the British Paediatric Association in a professional survey published in 1972 as Paediatrics in the Seventies (2) A year later the Government responded, and their approach was reflected in the composition of the committee established to pursue the national inquiry they proposed

This consisted of twenty members 3 parents, 2 teachers, 1 educational psychologist, 1 general practitioner, 1 health visitor, 2 social workers, 2 paediatricians, 1 child psychiatrist, 1 paediatric surgeon, 1 paediatric nurse, 1 school doctor, 1 dentist, 2 community physicians, 1 epidemiologist—with me as chairman

The Committee's brief was 'To review the provision made for health services for children up to and through school life, to study the use made of these services by children and their parents and to make recommendations to the Government' (3)

It reflected the strength and the limitations of Government thinking on the positive side a joint parent professional and inter professional approach, on the other an emphasis wholly on services Yet services should be determined by needs and needs are conditioned in large measure by the society in which they arise The Committee therefore widened the question to What is the character of British society today and what is the child s place in it?

Let me begin with the anatomy of the situation In 1976 there were 49 million persons in England and Wales, of whom 12 75, 26%, were children under 16 They lived in 6 5 million families, 92% living with their parents and 8% with their parents and with grandparents or other relatives

Family Life

Despite difficulties of definition, the family is still for us the primary guardian of children's

* The Rosen von Rosenstein Lecture delivered to the Swedish Paediatric Association 18 May 1979

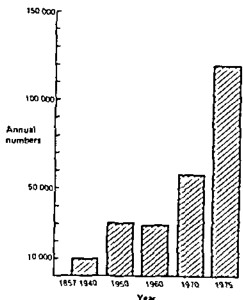


Fig 1 Divorce in England and Wales 1857–1975

health, and we have found no better way to raise a child than to reinforce the ability of his parents, whether natural or substitute, to do so Does the contemporary picture of British families suggest that our belief is justified? The general answer is *yes*, but this needs critical examination I begin with marriage

Marriage

Marriage is popular, and more than 90% of women have married by the time they reach the end of the child bearing period And although it is taking place at a younger age—in the last 25 years the mean age for men has fallen from 27 to 24 and for women 24 to 22—this has not been followed by an increase in family size Present trends suggest stabilisation at a completed mean family size of 4, two parents with two children We can therefore expect stability of the child population at present levels by 1981 A million fewer children than five years ago could make possible improvement in services without increasing costs In a structural sense the picture of the contemporary family is encouraging, but the quality is more varied and more difficult to interpret

Divorce

If marriage is popular, divorce is popular too, and it is taking place earlier in the marriage (Fig 1) The parents needs are loudly proclaimed, but little is known of the long-term effects on the children Since adverse experiences in childhood can have a persisting effect in adult life this carries important implications for the health and happiness of children and the stability of family life which our society is unwilling to face

One parent families

The family is not always complete In Britain in 1976 1 4 million children were living in 750 000 one-parent families In 10 per cent of the fatherless families the mother was unmarried

Poverty

Poverty is a relative term and opinions differ on how to measure it One measure is the official level of need which qualifies an individual to receive 'supplementary benefits' On this standard it was estimated in 1974 that 504 000 families with 1 114 000 children were living at or below the contemporary poverty level

Working mothers

40% of mothers aged from 25–34 are working outside the home

Such mothers are pressing for better care of their pre-school children, but Britain has an incomplete and untidy network of provision child minders, play-groups, nursery classes and nursery schools In a recent study only 1 in 3 children under 5 was receiving any 'day-care' but a further third had mothers who would have used these services if they had been available We are failing to provide care learning opportunities and continuing health surveillance for children under 5 whose mothers go out to work, or intermittent relief for those who remain at home

Parental illness

There is a considerable volume of chronic illness in parents, in our Family Studies in Newcastle (4) 12% were ill for long periods In

addition 14% of mothers suffered for many years from excessive weariness and dejection (5) In 1975 Brown (6) in London showed that this malaise of motherhood varies with social circumstances 25% of working class mothers had such symptoms compared with 5% of middle class mothers, and the figure rose to 42% in working class mothers with a child under 5

A multiracial society

In the 1950 s economic expansion led to the invited arrival of ethnic minorities from the new Commonwealth West Indians, Indians and Pakistanis now constitute between 3 and 4% of the total population, and clustering in particular localities, may raise that level to 25% The provision of services for their children is complicated by the fact that they live in the poorer parts of cities where services are least, and by differences of language and culture

Children unable to live at home

Where natural family care is lacking or seriously inadequate children may be cared for by local authorities In 1974 in England and Wales there were 31 000 children with foster parents and 45 000 in local authority or voluntary residential care These include 8 000 living in long-stay hospitals For them the hospital is the only home many of them will ever know, and by the end of the century many thousands of children will have spent their entire child hood in a hospital setting

The disadvantaged

Disadvantage is a term widely used to express the sum of adverse influences affecting children in a community The term has no precise meaning but we know what it implies Children who grow up in poverty or squalor, whose homes are grossly overcrowded, who are physically or mentally handicapped or seriously neglected, who are discriminated against on grounds of race, language, colour or religion, whose parents are ill, or who quarrel incessantly, or who are absent—such children are in different ways 'disadvantaged' And the precise estimate of disadvantage is increasingly relevant to paediatrics as such children are more likely to suffer from physical illness or psychiatric disorder, to fail educationally and to become delinquent

Education

The school years, with opportunities to learn the delight and discipline of work and play, are a critical period in the achievement of a responsible and caring society In Britain we have been slow to study and to develop edu cational paediatrics—that is the concept of health for education and the paediatrics of teaching institutions If our first task is safer and happier childbirth, the second a comprehensive service for the under 5 s, then the third is a relevant school health service—in which specifically trained paediatricians, in partnership with teachers, school nurses, psychologists, and parents, work within the school dealing with all aspects of 'educational failure to thrive'

Adolescence

Adolescents are among the healthiest and the most disturbed of our citizens The range of hazards to their health is wide accidents, sexual confusion, pregnancy (especially the children who are bearing children—1 600 births, and 3 000 abortions in girls between 11 and 16 in 1974), sexually transmitted diseases (600 new cases of gonorrhea in adolescents under 16 and 4800 between 16 and 17 annually), drugs, mainly tobacco and alcohol, psychiatric disorders, and suicide In need of help they are least willing to accept it—especially from conventional authority—and we have failed so far to devise generally acceptable services for them

Government

I have considered some aspects of our changing society which have a bearing on the health of children The ultimate responsibility lies with the Government, and what is their atti-

Table 1 *The increase in tax and national insurance (%) 1964/65–1978/79*

Household	Income				
	Below average earnings	Average earnings	A E ×2	A E ×5	A E ×10
Single person	54	36	27	70	65
Married couple	76	51	30	69	65
Married couple with 2 children	186	144	56	76	70

tude to children? Here nothing is more dangerous than a cherished illusion, the politician's belief that we love our children and are doing everything possible for them This was first questioned in Britain in 1948 by the late Richard Titmuss (7), and I repeated the analysis of direct taxation as it affects married families with and without children on which his misgivings were based

Although the 'child benefit', an untaxed cash payment to mothers, has increased this year, the penalising of families through direct taxation has not only persisted but increased (Table I) This is difficult to explain Titmuss suggested that in certain respects social attitudes were hostile to the family, and the general tendency of Government was to consider the nation as a collection of individuals rather than families In keeping with this, is the fact that from 1948 to 1972, children's services were never identified separately in our National Health Service, and expenditure was related to the type of service, not to specific groups, such as children or the aged, who require specific services In 1973, health expenditure on the 26% of child citizens was 9% of the total spent on health, and without implying that the allocation should be on a pro-rata basis, the size of the difference suggests that children may not be getting an appropriate share

III HEALTH SERVICES FOR CHILDREN

Partial social analysis can be misleading, Yet because the origins of childhood illness are found increasingly in social pathology this cannot be avoided It is widely agreed that not more than 10% of the improvement in health in the last hundred years has been due to medical treatment The main determinants were a rising standard of living, a more ample income, improved housing, better nutrition and wider education. These general forces are still important, but in the last thirty years a more exact and informative physiology and pathology have led to specific therapy and specific prevention which are now making an increasingly significant contribution

The Delivery of Health Services in Britain

A changing society is reflected in the changing pattern of its illness, and this in turn calls for modified services to meet new needs Before indicating the proposals which seemed right to the Child Health Services Committee the context of change must be defined This can be done most readily by asking these questions Are children as well as they could be? Are services as effective as they should be? Are parents as satisfied as they have a right to be? Are the professions involved as well trained as they need to be?

Are children as well as they could be?

This century has seen a notable improvement in the health of children in the developed world Not only are more children surviving, but they are taller, better nourished, freer from infective illness, better educated and probably happier than at any time in our history

The gaps

So why is good, not good enough? Because there are worrying gaps which must be faced

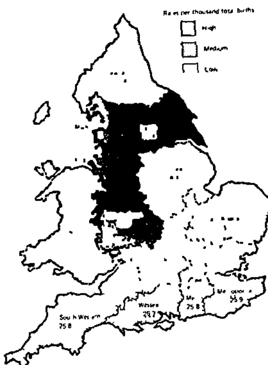


Fig 2 Infant life wastage (still births and deaths under 1 year) 1973 in England and Wales

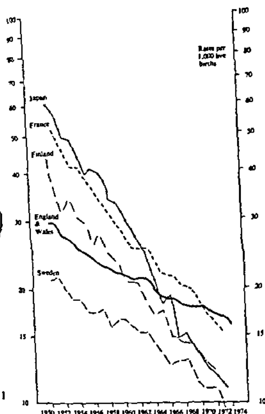


Fig 3 Infant mortality rates in selected countries 1950–1974

Survival

One of Rosen's strongest concerns was to lower the high level of infant deaths in the Sweden of his day I too will begin with survival We lose 18 000 lives every year in England and Wales at birth and in the first year, and a further 6 000 between 1 and 18

Infant life wastage is spread unevenly, and therefore inequitably, over the country 23/1 000 total births in East Anglia 33/1 000 on Merseyside (Fig 2) And if we want to identify the ultimate foci of mortality we must take our analysis down to neighbourhoods and compare mortality in the most advantaged and the least advantaged community wards

And when we turn to international comparisons the situation is also unfavourable (Fig 3) In the league table of the fifteen most effective countries in terms of perinatal and infant mortality, England and Wales are now eleventh, Japan, Finland, France, way behind us 15 years ago, are now ahead, and Sweden remains the pace-setter for all

Illness

The pattern of childhood illness is changing There is still acute illness and injury, but chronic illness and handicap are moving to the centre of the stage (Table 2) Malformations affect 1 in 50 pregnancies In the Isle of Wight, an advantaged community, 1 in 7 (8) children, and in Deptford, a disadvantaged London Borough, 1 in 4 children are handicapped [1] If we consider psychiatric disorder alone, at least a million children will be affected in England and Wales this year to a degree which will interfere seriously with education and with personal and family happiness Most testing of all are the illness and injury in children which

[1] A handicap is a disability which for a substantial period or permanently retards distorts or otherwise adversely affects normal growth development or adjustment to life

Table 2 *Children's health problems in England and Wales now*

Defective fetal growth
Hazards of birth
Bodily malformation
Illness
Injury
Physical or mental handicap
Psychiatric disorders
Educational failure
Social handicap
Parental deficiency

reflect the tensions and breakdown of family life

We must also recognise a change in parental expectations, expectations not only of more effective care but of a new type of relationship with professionals, not the episodic diagnosis and treatment model appropriate for acute illness, but the continuity of support, taken to the family and pioneered by the health visitor (public health nursing) service These changes in illness and relationships will call for new forms of paediatric education to enable doctors, nurses, therapists and administrators to cope with them

Knowledge

Medical knowledge is increasing more rapidly than its application This which all of us find difficult to admit, is seen in Britain especially, but not exclusively, in general practice It would not be too sweeping a statement to say there is hardly a disease in childhood which could not be better treated than it is at present

Prevention—treatment

For historical reasons, which no longer apply treatment and prevention are seen in Britain as different activities and practiced by separate services We want to overcome this unreal division so that prevention and treatment are seen as complementary parts of a single process and, as far as possible practiced by the same person

Parents and professionals

As a Government Committee to whom any one could send evidence or comment we heard more loudly than in our professional contacts the growing murmurs of parental dissatisfaction Parents find our services confusing, inaccessible, and frustrating to use They also find doctors difficult to talk to and are increasingly unwilling to be treated as passive bystanders in decisions about their children's health

The present services

Britain has three separate overlapping and poorly connected services A treatment service for those who seek it, in general practice a health surveillance disease prevention service offered to parents and children in the child and school health service a hospital centered, community deficient, paediatric service

IV WHAT ARE OUR OBJECTIVES FOR THE FUTURE?

We should move towards a single comprehensive service providing treatment of illness developmental oversight parent guidance prevention of disease and the promotion of health

This should be child centered and parent involved

The service should be equitably distributed throughout the country

The service should reach all children

All who work in the service should understand the development of children and be trained to meet their personal as well as their technical needs

This single service will call for increasing collaboration between parents medicine nursing education and social work

V HOW CAN WE ACHIEVE THESE OBJECTIVES?

Which road will best lead to the comprehensive child health service we seek and who will be the road builders The options are these Strengthen the present system create a sepa

rate paediatric service, develop paediatric practitioners in general practice, achieve a measure of paediatric specialisation within the context of family medicine and general practice

We recommended the last and the reasons are set out elsewhere (3)

Unlike Sweden, and indeed most countries in Europe except Holland, we shall for the present leave the primary care of children in the hands of general practitioners, rather than paediatricians This is based on principle and necessity, paediatrics and general practice are family disciplines, and there are 25 000 general practitioners in Britain today

By a recent act of Parliament all general practitioners will, in future, have vocational training in child health, and play an increasing role in preventive work particularly for children below school age Hopefully this will lead to a second stage in which one or two general practitioners, depending on the size of the group will accept a longer training in paediatrics and with it, responsibility for ensuring that all children in the practice receive regular developmental surveillance immunisation, and other preventive care They may or may not provide this themselves, would give illness care only to their registered children, and would have sufficient adult responsibilities for credibility with parents and the general work of the practice We called them general practitioner paediatricians or 'GPPs'

Health visitors (community nurses) are central to the service we seek They take the service to the home and their attachment to general practitioners to form the nucleus of the primary care team is the most significant development in the health care of children since the introduction of the National Health Service in 1948 However, if the needs of children are to be effectively met the primary paediatric emphasis, which was once the essential feature of their work, must return

At present less than half the child population under 5 attend child health clinics Since health visitors became family health visitors, covering all ages, the proportion of their time given to children under 5 has diminished and is now less than 60% Again, for a variety of reasons, a substantial number of children, as many as 1 in 10 in central city areas, are not registered with a general practitioner and their contact with the service is at best capricious and at worst non-existent We therefore suggested that some health visitors should accept a major responsibility for children, with the title child health visitors and the following pattern of work

They will share with midwives in antenatal preparation for parenthood In association with the 'general practitioner paediatrician',[1] they will provide health surveillance for all the children registered with the practice When ill children can remain at home they will guide the mother in nursing care

They will accept responsibility for ensuring that in a defined 'parish' contact is made with all children, and the parents persuaded to register with a general practitioner

Within this 'parish' too they would visit and advise Day Nurseries, Play Groups and other Child Care associations and activities

They will provide professional comradeship for specialist nurses working in schools in the area of the practice

Paediatricians

The majority will continue to safeguard the standards of hospital care A growing minority will take paediatrics into the community of which the hospital is a part and specialise in preventive, educational and social paediatrics

The community paediatrician

For us these are a new variety not a new species of paediatrician The areas of paediatrics for which they will be trained and accept responsibility are broadly the care of the handicapped and educational and social pae-

[1] The professional establishment [illegible]
a fair abbr[illegible]
evolve and [illegible]

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diatrics A particular setting for their work will be the Child Development Centre and the District Handicap Team They will act as 'consultants' to the primary and secondary schools in their district, and as school doctors for schools concerned with special education Their consultant responsibilities will also include the district social work team, and adoption and fostering agencies

They will be involved in appropriate clinical care within the district hospital, with a special responsibility for the teaching of social paediatrics

The District Handicap Team

The fourth agent of change is the District Handicap Team, working in and from the Child Development Centre in the District Hospital The core members will be a community paediatrician, specialist health visitor, specialist social worker, principal psychologist and remedial teacher Each handicapped family would look to one member of the team for guidance and would have direct access to this person Such teams are an essential response to the complex and often insistent needs of such families and the professional partnership required to deal with it

The Children s Committee

The central problem of change is maintaining momentum and building in machinery which can modify change as it proceeds The question asked by the Child Health Services Committee at the end of its inquiry was Whose job will it be to see that any of our recommendations that are accepted are actually carried out? Not just as elegantly worded circulars of advice and direction but actually in clinics hospitals, schools and homes, and in the spirit we intended There is no existing group we can ask to take this on The Government responded by establishing a national, independent, advisory body, the Children s Committee for this purpose It consists of 15 members, appointed not as representatives of particular professions but to ensure that the experience and expectations of parents and the insights of education, social work, and children's medicine would be available Its authority lies in the fact that children have special needs which they cannot articulate for themselves and therefore society has a duty to ensure that these are identified and cogently represented Children now have an advocate at the highest level with direct access to the Secretaries of State

A central task will be the preparation of a triennial review of the state of children s health to be laid before Parliament In a democratic society Parliamentary scrutiny is the surest guarantee that the rights of minorities or citizens with special needs will be upheld

Epilogue

I conclude by turning again to the responsibilities of paediatrics today and tomorrow To record them is to see how demanding they will be in study and in practice Low birth weight, birth hazards, malformations, handicap (physical, mental, social, multiple), illness (acute, recurrent, persisting) psychiatric disorders, accident, cancer, family dysfunction and child abuse, failure to thrive (somatic, educational), adolescent disorders and diseasee, widening prevention, educational paediatrics and education for health

Do we find this depressing or exciting? There are timorous voices in Britain who suggest that the major contribution of paediatrics has been made and that in future it will be a contracting discipline Those who think like this have not looked up from the esoteric problems of their hospital practice, much less moved out into the community to which their hospital should be open And they have certainly never come face to face with the daunting problems of child health in the Third World My reply to them is that on the commitment and courage of paediatrics in the remainder of this country the health of the world s children will largely depend

LYMPHOCYTE SUBPOPULATIONS IN NEONATES, YOUNG CHILDREN AND ADULTS AS DETECTED BY SIX CELL SURFACE MARKERS

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Acta Paediatr Scand, 69 13, 1980 —In this study, six surface marker tests were performed on lymphocytes from normal individuals of three age groups neonates, children from 0 to 2 years of age, and adults Determined were the proportions of T cells binding sheep red blood cells, of B cells carrying surface immunoglobulins or binding mouse red blood cells, and of lymphocytes bearing receptors for IgM, for IgG or for complement The T cell percentage appeared to increase with the age, the percentage of B cells was highest in the children's group, as determined by both marker tests Neonates had a much lower proportion of IgM receptor bearing cells than older individuals The variation in the percentages of T cells and of IgM receptor bearing cells in the young age groups was relatively large as compared to adults The significance of these observations is discussed, they may be of value for the proper evaluation of results obtained in diagnostical tests on neonates and young children

KEY WORDS Neonates, young children, lymphocyte surface markers, lymphocyte subpopulations

During the last decade, the identification of blood lymphocyte subpopulations by cell surface markers has become a widely used diagnostic tool in clinical immunology (10, 35, 41) The most generally employed and most specific markers are the affinity for sheep erythrocytes (E_S) displayed by T cells (23) and the surface bound immunoglobulins (sIg) which occur on B cell (40) Furthermore, tests for complement receptors (CR) (30) and for the Fc part of IgG (IgG-FcR) (9) are often performed, these markers are, however, not absolutely restricted to either T or B lymphocytes

By now, a vast body of normal values has been collected for these marker tests (cf Ref 10) but most reports are confined to adults, studies in children are less numerous (6, 13, 22 33, 34, 39) and usually, only E_s-binding, sIg and/or CR have been tested for Therefore, we have studied the occurrence of E_s-binding T cells, sIg bearing B cells and of lymphocytes bearing CR or IgG-FcR among blood lymphocytes from neonates and children up to two years of age Furthermore, we determined the frequency of lymphocytes bearing two more recently identified surface markers on which no information existed for the youngest age groups at all These are the affinity for mouse erythrocytes (E_m) and the receptor for the Fc part of IgM (IgM FcR) which occur on B cells (19, 37) and mainly on T cells (17, 27, 28), respectively The results obtained are being compared with the adult values

MATERIALS AND METHODS

Subjects of the study (*a*) 10 apparently healthy full term neonates delivered at the Department of Obstetrics of the University Hospital (*b*) 24 children of ages ranging from 1 month up to 2 years who were admitted to the University Children s Hospital to undergo a variety of surgical interventions none of which pertained to dis

Table 1 *Percentages of blood lymphocytes bearing different surface markers*
E_s affinity of T cells for sheep erythrocytes IgM FcR=receptor for the Fc part of IgM sIg=surface immunoglobulins on B cells E_m=affinity of B cells for mouse erythrocytes IgG FcR=receptor for the Fc part of IgG CR=complement receptor

Marker	Adults Mean	S D	Range	*n*	Children Mean	S D	Range	*n*	Neonates Mean	S D	Range	*n*
E_s (T)	69 3[a, b]	9 2	53–88	100	61 9[a]	7 0	49–73	24	56 9[b]	13 1	39–83	10
IgM FcR	58 8[c]	7 4	45–68	20	59 4[d]	17 9	14–86	23	21 8[c, d]	14 5	4–47	9
sIg (B)	9 2[e]	4 2	3–24	87	13 1[e]	4 3	5–22	24	11 7	4.5	6–19	10
E_m	5 0[f]	2 1	1–11	43	6 7[f]	3 7	2–15	24	5 3	1 6	3– 8	10
IgG FcR	25 9[g]	8 2	12–45	41	21 4[g]	5 3	13–32	24	24 5	9 5	13–41	10
CR	25 8	8 8	10–45	64	24 5[h]	4 6	17–32	24	20 5[h]	5 9	11–33	10
Blood lymphocyte count/mm³	2 200	550	1 050–3 950	100	6 300	1 900	2 150–10 700	24	4 450	850	3 300–5 750	10

Level of significance of differences between mean values are indicated with the following letters (if p is <0 05)
[a] p<0 002 [b, c, d, e] p<0 001, [f, g] p<0 02 [h] p<0 05

age blood lymphocyte counts in children and neonates were considerably higher than in adults, the absolute numbers of the various lymphocyte subpopulations were also higher in the youngest age groups The only exception was the IgM-FcR bearing lymphocytes, whose percentage was much lower in neonates than in children and adults

There were some further differences between the age groups in terms of percentages First, adults had a higher percentage of E_s-binding cells than newborn infants and children Secondly, the children had a higher percentage of B cells than adults, both in terms of sIg bearing lymphocytes and as E_m-binding lymphocytes Thirdly, the proportion of IgG-FcR bearing lymphocytes in children was lower than in adults, whereas in neonates the percentage of CR-positive lymphocytes was lower than in the other groups

The occurrence of two surface markers, notably the affinity for E_m and the IgM FcR was investigated to a somewhat further extent Fig 1 shows a scatter diagram of paired values for the E_m-binding and sIg bearing B cells It can be seen that the percentages of E_m-binding cells were almost always lower than the percentages of sIg-positive B cells scored In children and adults, the percentages obtained with both tests were positively correlated

In neonates, relatively few IgM-FcR bearing cells could usually be detected but in children, the mean percentage already equalled the adult value In Fig 2, the results obtained in the children's group have been plotted against the age Adult levels of IgM-FcR carrying cells appear to be reached quite early, only in some children younger than 6 months, are low percentages found. In addition the data for the

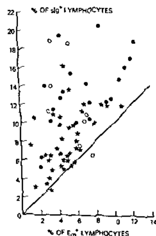


Fig 1 Relation between the percentage of blood B lymphocytes (sIg⁺) and ... cytes (E_m^+) ... ren (ages ... neonates (cord blood lymphocytes), r=0 49, not significant Line Points of percentual equivalence of both markers

orders having a known association with immunological disturbances (*c*) 100 apparently healthy adults (hospital personnal and blood bank donors) from 24 up to 58 years of age.

Blood sampling Blood samples were collected in siliconized tubes containing 10 I.U preservative-free heparine per ml of blood Cord blood, usually collected during the night, was stored at room temperature until processing (at most 8 hours later) Previous experiments had shown that this did not influence the outcome of the tests or the lymphocyte counts The other blood samples were taken between 8 and 10 a m , and in the case of the children, always prior to pre surgical medication These samples were processed immediately

Lymphocyte isolation Blood samples were diluted with 1 volume of Eagle's Minimum Essential Medium except for cord blood which was diluted with 2 volumes to improve the lymphocyte yields Lymphocytes were isolated by centrifugation of the dilute samples on Ficoll-Isopaque (5) Cord blood lymphocyte suspensions were always contaminated with red cells, these were removed by NH_4Cl induced lysis (32) The cells were washed three times with medium containing 1% w/v bovine serum albumin Lymphocytes and non-lymphoid leukocytes (mainly monocytes) were identified and counted in a counting chamber suing a Zeiss phase contrast microscope after diluting the sample with gentian violet in acetic acid (Turk's fluid) Lymphocyte counts on the blood samples were performed the same way This version of differential leukocyte counting and the conventional method had been extensively compared previously and shown to give identical results The present technique was chosen because it was simple, rapid and less subject to artifacts and errors The average lymphocyte yields were 40±20 (S D) % in neonates, 56±15% in children and 66±8% in adults In none of the age groups was a relation found between the lymphocyte yields and the outcome of any of the marker tests After counting, the cells were resuspended in the albumin containing medium at 2×10^6 per ml

Detection of monocytes When performing lymphocyte marker tests, it is essential to identify the monocytes which invariably contaminate the cell suspensions, particularly since monocytes carry IgG FcR and CR as well and may be false-positive for sIg in immunofluorescence Therefore, part of each lymphocyte sample was incubated with latex particles to mark the phagocytes The latex (aminolatex, Rhone-Poulenc, Courbevoie, France) had been labelled with rhodamine in order to visualize phagocytes within the rosettes formed with the indicator red cells used for the marker tests (see below) Details of the technique have been described elsewhere (16) After the latex incubation the cells were spun over a layer of 5% bovine serum albumin solution in medium at 37°C This procedure removed the free latex, most of the plate let contamination and also cytophilic IgG which might give false-positive results in the detection of SIg (24, 25, 42) The tests for sIg, IgG FcR and CR were carried out with lymphocytes thus treated, under a fluorescence microscope adjusted for green incident light Cells containing red fluorescent latex particles were excluded from the countings

The net percentage of lymphocytes binding E_s, E_m or bearing the IgM-FcR was determined as follows After completion of the counting, an equal volume of Turk's fluid was added to the test tube and the percentage of monocytes and lymphocytes was determined using phase contrast The net percentage of lymphocytes having a given marker was then calculated by the formula

$$\text{net } \% = \frac{\text{no of positive leukocytes/100 leukocytes}}{\text{no of lymphocytes/100 leukocytes}} \times 100$$

This was permitted by the previous observation that monocytes do neither have affinity for E_s or E_m nor bear IgM-FcR, as determined by the present techniques (16)

Indicator erythrocytes for the rosette tests E_s, E_m and erythrocytes sensitized with complement, IgG or IgM were prepared batchwise and stored as small aliquots for single use, at −85°C suspended in hydroxyethyl starch (18)

E_s rosette test for T lymphocytes This test was performed essentially as described by Brown & Greaves (4) Rosettes (cells with 3 or more firmly bound E_s) were counted in a haemocytometer after supravital staining of the leukocytes with Brilliant Cresyl Blue 200–400 leukocytes were scored as free or rosetting

Demonstration of sIg as a marker for B lymphocytes sIg were demonstrated using a fluorescein conjugated goat IgG anti human IgG-F(ab')$_2$ preparation with reactivity against κ and λ chains 200–400 lymphocytes were scored as positive or negative under alternate blue incident and auxiliary illumination using a Leitz fluorescence microscope equipped with 4× eyepieces, a 100× plan apo objective and a 100 W Hg lamp

Complement receptors CR were demonstrated using E_s sensitized with anti-E_s antibodies of the IgM class and mouse complement, technical details have been described elsewhere (16, 44)

IgG-Fc receptors Cells bearing IgG-FcR were enumerated by rosette formation with ox red cells, coated with IgG-class anti red cell antibodies (16, 21)

IgM-Fc receptors These receptors were demonstrated using ox red cells sensitized with antibodies of the IgM class, essentially as described earlier (17) However, in the present study the test was performed with lymphocytes that had been incubated overnight at 37°C in a medium devoid of IgM (28)

E_m rosette test Lymphocytes with affinity for E_m were detected the same way as E_s binding cells but the incubation was performed at 4°C

Statistical analysis The results obtained for the three age groups are expressed as arithmetic means ± 1 standard deviation The level of statistical significance was calculated using Student's *t*-test Relations between the outcome of two marker tests in individual donors were evaluated by the method of least squares *p*-values over 0 05 were considered as not significant

RESULTS

The results of the tests for lymphocyte surface markers are given in Table 1. Since the aver-

Table 1 *Percentages of blood lymphocytes bearing different surface markers*

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	Adults				Children				Neonates			
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E_m	5 0[f]	2 1	1–11	43	6 7[f]	3 7	2–15	24	5 3	1 6	3–8	10
IgG FcR	25 9[g]	8 2	12–45	41	21 4[g]	5 3	13–32	24	24 5	9 5	13–41	10
CR	25 8	8 8	10–45	64	24 5[h]	4 6	17–32	24	20 5[h]	5 9	11–33	10
Blood lymphocyte count/mm³	2200	550	1050–3950	100	6300	1900	2150–10700	24	4450	850	3300–5750	10

Level of significance of differences between mean values are indicated with the following letters (if p is <0 05) [a] p<0 002 [b, c, d, e] p<0 001 [f, g] p<0 02 [h] p<0 05

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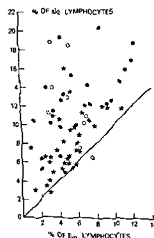


Fig 1 D[illegible] phocyte [illegible] cytes (E [illegible] ren (age, 1 month – 2 years) r–0 68 p<0 001 ○ 10 neonates (cord blood lymphocytes) r=0 49, not significant Line Points of percentual equivalence of both markers

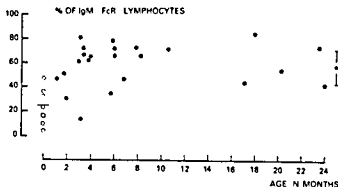


Fig 2 Percentages of IgM FcR bearing lymphocytes in 23 children (●) in relation to their ages O values for neonates ★, mean value for adults with 2×S D indicated

youngest children vary widely, as compared with children above 6 months of age and adults Moreover the mean percentages of E_s-binding T cells and IgM-FcR positive lymphocytes of the children's group were almost equal This was interesting since this marker was originally found (in adults) only on a subset of T cells (17, 27, 28) and later on some B cells (11, 31) When the percentages of E_s-rosetting and IgM-FcR bearing lymphocytes are plotted (Fig 3), it indeed appears that in some children, much more IgM-FcR positive cells than T lymphocytes were scored, which contrasts with the observation in neonates and adults

In order to obtain information on the reproducibility of the marker tests in time, longitudinal observations were made in several adults, over periods of 6 months to two years (results not shown in detail) For the majority of the markers (exceptions CR and IgG FcR), the longitudinal variation per individual was found to be less than the variation within the total group of adults Furthermore in a given donor, ther percentage of lymphocytes bearing a certain marker appeared to be more constant than the absolute number of blood lymphocytes

DISCUSSION

Many forms of immunodeficiency become manifest early in life and therefore, screening of the *in vitro* properties of lymphocytes from children is frequently indicated Because the enumeration of lymphocyte subpopulations is part of the diagnostic arsenal it is necessary to have control data available on the relevant age group Our results show that the surface marker pattern of the blood lymphocytes of young children and neonates indeed differs from adults in some respects

The observation that cord blood contains a relatively low proportion of E_s-binding T cells has also been reported by others (2, 3, 6, 8 36, 43) The same is true for the observation that the proportion of these cells gradually increases after birth to reach adult values after at most a few years (2, 3, 13) The ranges of E_s-binding cells among cord blood lymphocytes were larger than those of the other age groups, this, too, has been observed by others (6, 43) Because these values were apparently not related to the time of storage of the blood or to the lymphocyte yields, these variations may well reflect "physiological" differences between individuals whose immune system is still partly immature and whose lymphocyte recirculation has not yet reached a steady state The variations may even be the larger since the average E_s-binding capacity of *all* cord blood lymphocytes appears to be rela

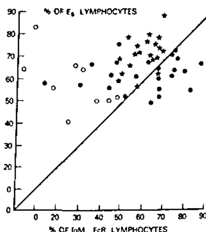


Fig 3 Relation between the percentage of blood T lymphocytes (E_s^+) and lymphocytes bearing receptors for the Fc part of IgM (IgM FcR$^+$) ★ 20 adults $r=0\ 59$ $p<0\ 02$ ● 23 children (ages 1 month – 2 years) $r=0\ 17$ not significant O 9 neonates (cord blood lymphocytes) $r=0\ 57$ not significant Line points of percentual equivalence of both markers

tively low and/or because a T cell fraction with a high affinity for E_s is present in a low number (15, 26 43) Also, a seizable number of cord blood lymphocytes though reactive with an anti T cell serum fails to form rosettes with E_s (8) They might be immature T lympho cytes because during foetal ontogeny, T cells reactive with anti T cell serum but failing to form E_s rosettes are more numerous and probably appear earlier than those bearing both markers (1) These observations point to the existence of qualitative differences between T cells in the blood of neonates and adults, apart from the obvious quantitative difference

The low, though variable, percentage of IgM FcR bearing lymphocytes in cord blood indicates that the composition of the T cell population differs from adults also in terms of subsets T cells bearing IgG and IgM FcR in adults show quite different kinetics of stimula tion when cultured with various T cell mito gens (29) Thus the large individual variation in the *in vitro* responsiveness to the same mito gens which was found in cord blood lympho cytes (38) might be related to the variation in the number of IgM FcR bearing lymphocytes between individuals in the youngest age group

Apparently, the percentage of IgM FcR bearing cells increases rapidly after birth and in about half of the children studied it was even higher than that of T cells Initially, this was a somewhat puzzling observation which had to be explained either by assuming that in these individuals many T cells were simply not being identified by the E_s rosette technique used or that B cells and/or other lymphocytes participate in the rosette formation with IgM sensitized ox erythrocytes Unpublished data obtained in our laboratory with isolated non T lymphocytes from children and a dults have indeed indicated that the latter may be the case Meanwhile, the occurrence of IgM FcR on B cells has also been found by others (11, 31) In view of these observation it can be concluded that the estimation of T cells having IgM FcR is best performed with iso lated T cells

The data obtained for the frequency of B lymphocytes are somewhat difficult to com pare with those of others since in the present study, care was taken to make the lymphocytes shed any cytophilic Ig from their surface (by incubation at 37°C) Disappearance of such passively absorbed Ig by incubation at 37°C (24, 25, 42) has also been documented for new born infants (34) As a consequence of this treatment, our percentages of sIg bearing B cells are relatively low as compared with earlier data from other groups In spite of this, our data agree with these in that newborns have a higher percentage of B cells than adults (2 6 8 39) whereas the highest percentage of B cells is found in children of ages between 0 and 2 years (39) The E_m binding lymphocytes follow a similar pattern Because the average E_m binding cell might be younger than the B lymphocytes lacking this marker (20) they could also have different circulatory proper ties This might then explain the lack of nu merical relation between the cells bearing these markers in neonates

The outcome of the tests for IgG-FcR and CR showed some differences between the age groups However, any speculation as to the significance of such differences is thwarted by the occurrence of these markers on lympho cytes of diverse ontogenetic origin (i e T cells, B cells and "null" lymphocytes) (7, 12, 14, 16) The finding that during a simple over night incubation many cord blood lympho cytes begin to express IgG FcR (43) indicates that unexpected and unexplained *in vitro* (?) events may interfere with the appropriate interpretation of the data as well

The differences between individuals were quite large in some tests which limits their application for diagnostic purposes Neverthe less, in such cases (e g the E_m rosette test) the ratios of the numbers of cells bearing differ ent markers (e g sIg and affinity for E_m) may provide useful information, particularly when more will be known about the life history and functional properties of lymphocyte subsets In addition, the reproducibility of the tests for

individual donors was rather satisfactory for a test on living cells (cf ref 25) In an accompanying paper, results are given of the application of the six lymphocyte marker tests for the study of patients with various immunodeficiencies

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Submitted Jan 8 1979
Accepted July 1 1979

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Acta Pædiatr Scand 69 21–24 1980

NON SPECIFIC IMMUNITY IN NEPHROTIC SYNDROME

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ABSTRACT Yetgın S Gur, A and Saatcı U (Department of Paediatrics Children s Medical Center, Hacettepe University, Ankara Turkey) Non specific immunity in nephrot ic syndrome Acta Paediatr Scand 69 21 1980 —In order to explain susceptibility to bacterial infection in patients with nephrotic syndrome bactericidal capacity of polymor phonuclear neutrophils (PMN) and serum opsonic activity were studied The groups con sisted of 29 patients and 29 controls Bactericidal capacity was found to be statistically

globulin total lipid cholesterol age of patients and the duration of the illness When bac tericidal capacity was examined in five patients in remission it had returned to the normal level The results of this study suggest that bactericidal capacity and possibly serum op sonic activity are influenced by the nephrotic syndrome

KEY WORDS Nephrotic syndrome, bactericidal capacity, opsonic activity, polymorphonu clear neutrophil, *Staphylococcus aureus Escherichia coli*

The susceptibility of patients with nephrotic syndrome to bacterial infections is well known (12) While these infections generally occur as upper respiratory tract infections and broncho pneumonia they can sometimes be serious diseases such as peritonitis or sepsis (4 11 16)

Formerly edema being an excellent medium for bacterial growth was the usual explanation for these infections in the nephrotic syndrome Cellular and humoral immunity defects have recently been shown in this syndrome (5, 7, 9 13 15) but it is known that microphages and macrophages play an important role in the protection of the body against bacteria For this reason the bactericidal capacity of the polymorphonuclear neutrophils (PMN) in pa tients with nephrotic syndrome was evalu ated Serum opsonic activity was also studied

MATERIALS AND METHODS

Twenty nine patients with an age range of 3 to 16 years (9 girls and 20 boys) and 29 age matched controls were studied simultaneously In each patient the diagnosis of nephrotic syndrome was based on the presence of edema proteinuria (excretion greater than 2 g/m²/day) hypoalbuminemia (less than 30 g/l) hypercholesterolemia (greater than 6 2 mmol/l) and a renal biopsy was per formed on 24 of them Albumin globulin total lipid cholesterol BUN creatinin C3 Hb and WBC were determ ned

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serum opsonic activity against the same bac terium were investigated The bactericidal capacity of PMN on *Escherichia coli* and serum opsonic activity against E coli were likewise investigated in 10 and 12 patients respectively Bactericidal capacity studied in 5 patients in exacerbation was reexamined in the same 5 patients in remission

The bactericidal capacity was measured using the method of Quie et al (10) The bacteria were suspended in Hanks

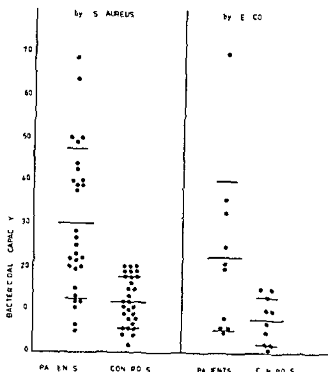


Fig 1 Bactericidal capacity of PMN in patients with nephrotic syndrome

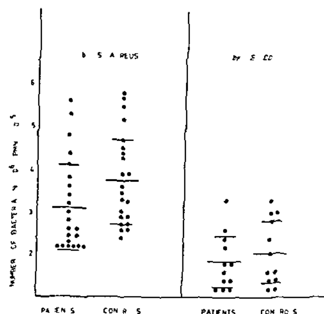


Fig 2 Serum opsonic activity in patients with nephrotic syndrome

Mononuclear contamination was 5–15% Sera were provided by 4 healthy persons and stored at −70°C for no longer than 20 days 0 5 ml of PMN suspension 0 4 ml diluted serum and 0 1 ml bacteria suspension were mixed *providing 2–3 S aureus per PMN and a final concentra* tion of 10% serum The tubes were incubated at 37°C with an end-over end rotation

Samples (0 1 ml) were removed periodically at 0 30 60 90 120 min pellets were placed in distilled water for lysis and for determination of the total number of viable bac teria counted by the pour plate technique The per

med um containing the patient s serum to the number obtained from the normal PMN in a medium containing normal serum after 20 min incubation (3)

C3 values were determined by plate immunodiffusion technique (Behringwerke) in 12 patients

RESULTS

The bactericidal capacity of PMN on *S aureus* in patients with untreated nephrotic syndrome was significantly reduced ($p<0\ 001$) only 7 of the 29 (24 1%) patients had values within the normal range (Fig 1) The bactericidal ca pacity of PMN on *E coli* was also found de creased in 6 out of 10 (60%) patients (Fig " which was also statistically significant con pared to controls ($p<0\ 05$) Serum opsoni *activity was found to be decreased (Fig 2* but it was not statistically significant for eithe bacteria ($p>0\ 05$)

The values for bactericidal capacity an serum opsonic activity of 24 patients in whon tissue diagnosis could be obtained are shown in 6 groups Because each group had too fev

Table 1 *The evaluation of serum factors re lated to renal functions and hematologica data*

Data	Mean ± S E
Total protein	46+1 0 g/l
Albumin	22+1 0 g/l
Globulin	23+1 0 g/l
Cholesterol	9 5+0 5 mmol/l
Total lipid	13 13±0 9 g/l
BUN	3±0 1 mmol/l
Creatin n	60±55 μmol/l
C3 (B C)	18 3±7 8 mg/dl
Hb	127±3 0 g/l
WBC	8 7±5×10⁹/l

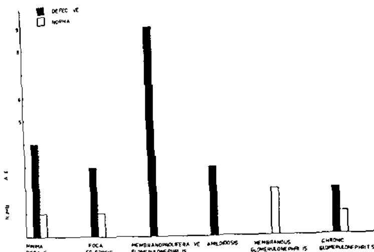


Fig 3 The evaluation of bactericidal capacity of PMN on *S aureus* in 24 patients with pathologic diagnosis

cases, they could not be evaluated separately (Fig 3) Serum albumin, globulin, total lipid, cholesterol, BUN, creatinin and C3 values were normal in all patients except one (Table 1) and no correlation between these factors, the age of the patients, the duration of the illness and the impairment of bactericidal capacity was present The hematological findings were normal in all

DISCUSSION

The protective role of the PMN in bacterial infections is well recognized (2) To explain the susceptibility to bacterial infections in patients with nephrotic syndrome, bactericidal capacity of PMN and serum opsonic activity were examined and reduction in these functions were shown Normal bactericidal capacity depends on the glycolytic activity, oxidative metabolic activity, production of H_2O_2, decreased intracellular pH and release of the lyzosomal peroxidative enzymes which are necessary for destruction of the bacteria by PMN (1) Hypoproteinemia in patients with nephrotic syndrome as in protein calorie malnutrition (14, 15, 16, 17) is thought to participate in the enzymatic function which may be responsible for the defective bactericidal capacity of PMN Although there is no correlation between bactericidal function and serum protein levels in this study, this correlation may be related to the amounts of leukocyte proteins Decreased acid phosphatase level of PMN in nephrotic syndrome (6) might be evidence for the above assumption Circulating immune complexes may also stimulate lyzosomal enzyme discharge of PMN and decrease bactericidal capacity by reducing the enzymes in PMN (18) which might then explain the defective bactericidal capacity in all patients with membranoproliferative glomerulonephritis in this study Decreased IgG and IgA levels have been reported in patients with nephrotic syndrome (5) The increased incidence of severe infections in these patients has been related to decreased serum opsonic activity as a result of reduced serum factor B (C3PA) (18) Although opsonic activity was found to be decreased in up to 50% of our patients it was not statistically significant However, the results of our study suggest that bactericidal capacity of PMN on Gram positive and Gram negative bacteria is reduced in patients with the nephrotic syndrome In 5 of the patients in remission, bactericidal capacity was re examined, and it was found to have returned to normal levels, which is a

further indication that the bactericidal capacity of PMN is influenced in nephrotic syndrome. The decreased bactericidal capacity of PMN in these patients is an additional explanation of their susceptibility to bacterial infections.

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Submitted Febr 27, 1979
Accepted May 7, 1979

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Acta Pædiatr Scand 69 25–30, 1980

IMMUNOCOMPETENCE IN OBESITY

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ABSTRACT Chandra R K & Kutty K M (Department of Pediatrics, Memorial University of Newfoundland, St John's Newfoundland, Canada) Immunocompetence in obesity Acta Paediatr Scand 69 25, 1980 —Thirty-eight % of obese children and adolescents showed a variable impairment of cell-mediated immune responses *in vivo* and *in vitro* and reduction of intracellular bacterial killing by polymorphonuclear leukocytes The obese group had a higher incidence of iron deficiency and moderately lower serum zinc concentrations Levels of serum immunoglobulins complement components C3 and C4, and numbers of T and B lymphocytes were comparable in the two groups Serum triglycerides cholesterol and lipoproteins were normal in all subjects Immunologic changes correlated with the presence of subclinical deficiencies of iron and zinc Therapy with these micronutrients for 4 weeks resulted in improvement in immunologic responses

KEY WORDS Obesity, nutritional disorder, cell mediated immunity neutrophils, lymphocytes delayed hypersensitivity, iron deficiency, zinc deficiency

The causal association of obesity with the occurrence of hyperlipidemia diabetes mellitus premature atherosclerosis and early death is widely accepted (15) Clinical epidemiologic and autopsy data suggest a higher incidence also of infectious illness in the obese compared with lean controls (16 21) Infection related mortality is increased (23) In dogs made obese by providing large amounts of food there was poor host response to challenge with *Salmonella typhimurium* or distemper virus, resulting in higher morbidity and mortality (18 27) Immunologic as well as non immunologic tissue factors for example reduced vascularity, physical inactivity and restricted respiratory movements may underlie such susceptibility of the obese to infection In genetically obese mice, there are significant alterations in cell mediated immune responses (8 8a) There are no published reports of a systematic evaluation of immunity function of obese human subjects We have observed impaired immunocompetence in a proportion of obese children and adolescents and found it to be related to associated subclinical deficiencies of iron and zinc

MATERIALS AND METHODS

Subjects

Twenty eight obese children and adolescents (age range 6–18 years 10 boys 18 girls) attending the outpatient Obesity Fitness Clinic or admitted as inpatients for evaluation and dietary management of overweight and 28 lean controls (age range 8–18 years 11 boys 17 girls) were studied Informed consent was obtained from the parents of the subjects and the research was reviewed and approved by the Medical Advisory Committee of the hospital The diagnosis of obesity was based on all of the following criteria physical appearance mean of subscapular suprailiac and triceps skin fold thickness greater than 20 mm measured with Holtain constant pressure callipers and weight for height index above the 95th per centile None of the subjects had any evidence of systemic disease or infection at the time of testing and they were not receiving any drugs including contraceptive pills

Delayed hypersensitivity

Cutaneous delayed hypersensitivity was assessed by injection of five common recall antigens into the epidermis and noting the size of induration 48 hours later Candida (Hollister Stier) purified protein derivative (Connaught) streptokinase streptodornase (Lederle) trichophyton (Hollister Stier) and mumps (Lilly) were used employing standard preparations and concentrations as recommended by the WHO Scientific Group on Immunodeficiency (26) The reaction was recorded as positive when the skin induration was 5 mm or more in diameter Eleven of the obese group and 14 of the controls had received B C G vaccine earlier in childhood

Table 1 *Serum concentrations of immunoglobulins and complement components PHA induced lymphocyte proliferation and bacterial killing capacity of PMN*

Data are shown as mean ± S E M Significance of differences was assessed by Student s *t* test

Group	No	IgG (g/l)	IgA (g/l)	IgM (g/l)	C3 (g/l)	C4 (g/l)	Lymphocyte stimulation index	Bactericidal capacity of PMN
Obese	28	10 86±2 04	1 96±0 23	1 02±0 14	1 56±0 12	0 27±0 04	30± 9	19±5
Control	28	11 91±1 56	1 71±0 21	1 15±0 12	1 45±0 13	0 33±0 04	107±15	2±1
p		NS	NS	NS	NS	NS	<0 01	<0 01

NS=not significant

Lymphoid cells

Heparinized peripheral venous blood was collected and mononuclear cells were isolated by Ficoll Hypaque density gradient centrifugation Cells were washed twice in Hank s balanced salt solution (BSS) and resuspended in RPMI 1640 containing 15% heat inactivated fetal calf serum Phagocytic cells were depleted by addition of lymphocyte separation reagent (Technicon) incubation at 37° for 30 min employing slow rotation and centrifugation at 400 *g* for 20 min Interface cells were washed twice with BSS and resuspended in RPMI 1640 at a concentration of 4×10⁶ cells per ml

T and B lymphocytes

Lymphoid cells were mixed with equal volume of 1% neuraminidase treated sheep red blood cells in 25% fetal calf serum The mixture was incubated at 37°C for 5 min centrifuged at 200 *g* for 5 min and incubated again at 4°C for 1 hour The cell pellet was resuspended by gentle agitation kept at 4°C for a further period of 10 min and examined for the proportion of cells forming rosettes with sheep erythrocytes and counted as T cells (5)

Cells bearing surface membrane immunoglobulin (smIg) were recognized by speckled or crescent shaped immunofluorescence on staining with fluorescein conjugated goat antiserum raised against human Igs (5) Mononuclear cells were incubated in culture medium at 37°C for one hour and then incubated with the conjugated antiserum After thorough washing the cell preparations were examined under a fluorescence microscope equipped with epi illumination Monocytes were identified and excluded by their ability to phagocytose latex particles smIg bearing lymphocytes were counted as B cells

Lymphocyte stimulation response

The ability of lymphocytes to proliferate and synthesize DNA *in vitro* in response to stimulation with phytohemagglutinin (PHA) was estimated by ³H thymidine incorporation (25) and the results were expressed as stimulation index (counts per minute in cell cultures containing PHA/counts in cultures without PHA) The unstimulated values were comparable in the two groups of subjects The maximum stimulated value was obtained after a PHA dose response curve The coefficients of intra and inter experiment variation were 11 and 15% respectively

Neutrophil function

The bactericidal capacity of polymorphonuclear leukocytes (PMN) was assessed by mixing PMN and *Staphylococcus aureus* in the ratio of 1 5 and incubating at 37°C in the presence of 20% pooled normal plasma At various intervals viable intracellular bacteria were estimated by surface colony counting on nutrient agar Results were expressed as ratio of viable bacteria at 140 min/similar count at 20 min The extracellular organisms were inactivated by penicillin streptomycin followed by washing the cells thrice prior to lysing them with distilled water

Immunoglobulins and complement components

Serum concentrations of IgG IgA IgM C3 and C4 were estimated by immunodiffusion in agar employing commercial plates (Hyland)

Nutritional biochemistry

Iron nutrition was estimated by red cell indices serum iron transferrin saturation and serum ferritin Ferritin was estimated by double antibody radioimmunoassay

Table 2 *Leukocyte counts and number of T and B lymphocytes*

Values are shown as mean ± S E M Significance of differences was assessed by Student s *t* test

Group	No	Neutrophils (10⁹/l)	Lymphocytes (10⁹/l)	T cells (%)	B cells (%)
Obese	28	3 8±0 4	3 0±0 3	67+5 1	15+2 4
Control	28	3 7±0 4	2 4±0 3	70±4 3	12±1 7
p		NS	NS	NS	NS

NS=not significant

Table 3 *Cutaneous delayed hypersensitivity*

Figures indicate the number of individuals who showed positive response Significance of differences was assessed by chi square test

Group	Total no	Candida	Purified protein derivative	Steptokinase streptodornase	Mumps	Tricho-phyton
Obese	28	14	4	12	9	9
Control	28	21	6	22	15	19
p		<0 01	NS	<0 05	<0 05	<0 05

NS = not significant

Serum zinc was measured by atomic absorption spectrophotometry

Serum cholesterol and triglycerides were estimated by enzymatic methods using commercial kits (Boehringer Mannheim) and lipoproteins by electrophoresis (Ames)

Treatment and reassessment

Individuals with deficiencies of iron (defined as transferrin saturation ≤16% and/or serum ferritin ≤18 μg/l) or of zinc (defined as serum zinc ≤70 μg/ml) were treated appropriately and the immunologic response was reassessed Each treatment period spanned 4 weeks and consisted of either iron given orally as ferrous sulphate 250 mg daily or zinc sulphate 100 mg by mouth daily In two individuals with combined deficiency of iron and zinc supplementation was provided with one substance at a time

Analysis

Statistical differences were analyzed by the Student s t test and paired t test In the case of delayed hypersensitivity response chi square test was used

RESULTS AND DISCUSSION

Of the various immunity functions assessed in the obese abnormalities were noted in two indices namely cell mediated immunity and phagocyte function Concentrations of serum immunoglobulins and complement components numbers of T and B lymphocytes, and phagocytic ability of PMN were comparable in the two groups (Tables 1, 2)

As a group, obese children and adolescents showed reduced cutaneous delayed hypersensitivity responses (Table 3) and impaired lymphocyte stimulation *in vitro* (Table 1) Bacterial killing capacity of PMN was decreased The obese group had a higher incidence of iron deficiency and lower serum zinc level (Table 4) Serum lipid profile was comparable in the two groups (Table 5) The immunologic and nutritional findings in the 11 subjects with altered leukocyte functions is shown in Table 6 Impaired lymphocyte and PMN activity was noted only in those obese individuals who had moderate micronutrient deficiencies None of the subjects was anemic PHA-induced lymphocyte proliferation was decreased in 7 and was associated with deficiency of iron, zinc or both Intracellular bacterial killing capacity of PMN was reduced in 6, and correlated only with iron undernutition Both iron and zinc deficiency, individually and together, was associated with reduced cutaneous delayed hypersensitivity and lymphocyte proliferation response *in vitro* Correction of nutrient deficits using appropriate therapy with either iron or zinc resulted in

Table 4 *Iron nutrition and plasma zinc concentrations*

Data are shown as mean ± S E M Significance of differences was assessed by Student s t test

Group	No	Transferrin saturation (%)	Serum ferritin (μg/l)	Plasma zinc (μmol/l)
Obese	28	13±2 7	15±1 9	8 3±1 1
Control	28	27±2 1	34±3 5	13 2±0 8
p		<0 05	<0 01	<0 05

Table 5 *Serum concentrations of lipids and lipoproteins*

Values are given as mean ± S.E.M. Significance of differences was assessed by Student's t test.
LDL=low density lipoproteins

Group	No	Cholesterol (mmol/l)	Triglycerides (mg %)	LDL (mg %)	Lipoproteins β (%)	Pre β (%)	α (%)
Obese	21	4.4±0.4	117±9	365±29	61±4	12±2	26±3
Control	15	4.1±0.3	129±8	329±37	63±5	11±2	23±2
p		NS	NS	NS	NS	NS	NS

NS=not significant

significant improvement in immune responses (Table 6).

There is no satisfactory explanation for the apparent paradox of micronutrient deficiencies seen in obese children and adolescents in this study. Dietary histories did not reveal any apparent cause(s) which may have led to such deficits. However, imbalance of nutrient intakes in the obese may well occur; many such individuals consume large amounts of fats and carbohydrates. The frequent prevalence of subclinical iron deficiency among certain at-risk groups such as female adolescents in North America is recognized (19). However, in that survey no correlation of nutritional deficiencies with body fat or any index of obesity was attempted. Atkinson and his colleagues (1) recently reported lower plasma zinc concentrations compared with levels in lean control subjects. Alterations in the plasma proteins that transport zinc were suggested as one possible cause of low serum zinc but no data were presented.

Protein-energy malnutrition increases susceptibility to infection and impairs immunocompetence (1, 4, 7, 17, 22). Recent studies have documented the adverse effects of the deficiency of a variety of specific nutrients on different facets of the immune system. These include iron, folic acid, pyridoxine, lipotropes, vitamin A and zinc (6, 9, 12, 14). The relationship of iron nutrition with immunocompetence has been extensively reviewed elsewhere (3, 9). More recently, zinc deficiency has been shown to impair several aspects of cell-mediated immunce response (6, 8b, 11). This is further supported by the occurrence of partial secondary immunodeficiency in patients with acrodermatitis enteropathica, a zinc-deficiency disorder (R. K. Chandra, unpublished observations). Administration of zinc clears the skin lesions and improves immunologic responses.

Serum lipids were comparable in the obese and lean groups in our study. There was no correlation between lymphocyte and PMN function and serum triglycerides, cholesterol or low density lipoproteins. In a separate study, we have looked at PHA-induced lymphocyte DNA synthesis employing leucocytes from two healthy adult donors and 50 hyperlipidemic serum samples. There was no correlation between lymphocyte stimulation response *in vitro* and concentrations of triglycerides, cholesterol or low density lipoproteins in the serum employed in cell cultures (R. K. Chandra, K. M. Kutty & B. Au, unpublished data). Thus we have failed to confirm the results of an earlier study (24) in which plasma from patients with primary hyperlipoproteinemia inhibited both spontaneous and antigen- or mitogen-induced ^{3}H thymidine incorporation by cultured mononuclear leukocytes; the suppression was attributed to the chylomicron and very low density lipoprotein fractions. Furthermore, it has been postulated that changes in lipid concentrations may mediate altered granulocyte

Table 6 *Lymphocyte and PMN function iron nutrition and plasma zinc concentrations before and after treatment*

Statistical significance of differences between means of values obtained before and after supplementation was calculated by paired *t* test except in the case of delayed hypersensitivity where chi square test was employed

Age (years)	Sex	Observation time	Delayed hyper sensitivity	Lymphocyte stimulation index	Bactericidal capacity of PMN	Transferrin saturation (%)	Serum ferritin (μg/l)	Plasma zinc (μmol/l)
9	M	Before treatment	0	12	22	14	17	11 6
		After iron therapy	+[a]	39	7	21	33	10 9
13	M	Before treatment	0	11	32	12	11	11 0
		After iron therapy	+	59	6	29	36	14 7
15	F	Before treatment	+	12	22	12	10	12 5
		After iron therapy	+	51	3	26	44	11 6
14	F	Before treatment	0	9	5	21	30	8 3
		After zinc therapy	+	47	4	23	32	14 8
14	F	Before treatment	0	11	21	14	11	7 5
		After iron therapy	0	43	4	29	37	8 1
		After zinc therapy	+	108	3	28	39	13 6
14[b]	F	Before treatment	+	17	6	15	16	13 2
		After iron therapy	+	110	3	38	40	12 4
15	M	Before treatment	+	8	5	26	41	5 5
		After zinc therapy	+	79	5	28	40	15 0
15	F	Before treatment	0	12	2	39	36	6 6
		After zinc therapy	+	103	3	35	39	18 1
16	F	Before treatment	0	5	7	27	39	4 9
		After zinc therapy	+	31	2	25	37	12 4
18	F	Before treatment	0	5	16	13	16	6 4
		After zinc therapy	+	29	19	15	17	11 9
		After iron therapy	+	87	2	32	29	13 5
19[b]	F	Before treatment	+	15	16	14	15	11 8
		After iron therapy	+	59	5	23	44	11 0
P			<0 05	<0 01	<0 05	<0 05	<0 05	<0 05

[a] Results were scored as + if the individual showed a positive reaction to one or more antigens and 0 if there was no response to any antigen

[b] From the lean control group These two girls were the only individuals among the controls who showed impaired immune responses and had reduced iron nutrition indices

function (10, 13) Incubation of PMN with saturated fatty acids inhibited chemotaxis and reduced bacterial killing Unsaturated free fatty acids stimulated leukocyte oxygen uptake and glucose oxidation However, in our study neutrophil dysfunction was unrelated to serum lipid concentrations, as also observed recently in another report (20)

Our data suggest that altered immunocompetence observed in a proportion of obese children and adolescents is causally linked with associated subclinical deficiencies of iron and [illegible] of nutritional status results in reversal of immunological dysfunction to normal

ACKNOWLEDGEMENTS

We are grateful to Greg Woodford and Bing Au for expert technical assistance Ferritin was estimated in a [illegible]

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Submitted June 5 1979
Accepted June 18 1979

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Acta Pædiatr Scand 69 31–35, 1980

D PENICILLAMINE, A NON BILIRUBIN-DISPLACING DRUG IN NEONATAL JAUNDICE

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ABSTRACT Brodersen, R , Lakatos L and Karmazsin, L (Institute of Medical Biochemistry, University of Aarhus Denmark and Pediatric Clinic, University Medical School, Debrecen, Hungary) D-penicillamine, a non bilirubin-displacing drug in neonatal jaundice Acta Paediatr Scand, 69 31, 1980 —D-penicillamine, a drug used clinically for the treatment of neonatal hyperbilirubinaemia, was tested for interference with the binding of bilirubin to human serum albumin by three methods 1) The peroxidase technique, investigating the effect of D-penicillamine on the equilibrium concentration of unbound bilirubin in a solution containing a molar excess of albumin, 2) the MADDS method measuring the concentration of vacant bilirubin binding site on albumin in a solution of pure albumin, or infant blood serum with added D penicillamine, and 3) injection of D penicillamine into Gunn rats and determination of any decrease of plasma bilirubin which would be caused by displacement of the pigment Results were negative in all cases Quantitatively, the doses of D-penicillamine used clinically cannot displace bilirubin from its binding to albumin The ameliorating effect on hyperbilirubinaemia in the newborn must be due to some other mechanism

KEY WORDS Neonatal hyperbilirubinaemia, D-penicillamine, albumin binding bilirubin displacement

D penicillamine (3,3 dimethylcysteine), given intravenously to newborns, significantly reduces the plasma bilirubin concentration or prevents the increase usually seen during the first few days of life (15–17) This treatment is especially effective in jaundice of haemolytic origin (2) such as ABO or rhesus incompatibility, and is used together with phototherapy (13) in several Hungarian clinics where it has largely replaced exchange transfusions

Any drug applied in the neonate should be tested for bilirubin displacing effect, exerted on binding to albumin Such testing seems particularly needed with a plasma bilirubin lowering drug since theoretically the effect might be explained by interference with the binding of bilirubin to albumin The possible effect of D-penicillamine on the binding of bilirubin [illegible] rats [illegible] We have performed more detailed investigations, using two other *in vitro* methods besides an *in vivo* test in Gunn rats

MATERIALS AND METHODS

D-penicillamine hydrochloride (Metalcaptase®) was a gift from Knoll AG Ludwigshafen a Rh/Berlin This sub-

[illegible]

[1] L L received a Danish government scholarship for research workers according to the cultural agreement with Hungary

procedure was defatted with charcoal in acid solution (9) tested for non oxidation of bound bilirubin (5) and the rate of dissociation of bilirubin from the primary binding site was measured and used for correction of the results (5)

The MADDS (*monoacetyl-diaminodiphenylsulfone*) method (4) was applied in a convenient micro modification (8) *using ^{14}C labelled MADDS* Studies were performed with solutions of human albumin (non defatted) and with neonatal serum

All *in vitro* tests were performed at 37°C pH 7 4 in sodium phosphate buffer ionic strength 0 15 M

Investigations in Gunn rats were carried out according to methods described by Johnson et al (12) Nathenson et al (19) and Ballowitz (1) Homozygous adult female Gunn rats weighing 180–250 g were treated with D penicillamine injected intravenously as a single dose (100–400 mg/kg in a 10% (w/v) solution) or with three doses at 1 h intervals (each dose 100 mg/kg) Control experiments were carried out with injections of isotonic sodium chloride solution the same volume as for injection of the drug and further groups of rats received intravenous doses of sodium benzoate (200 mg/kg) a moderately active bilirubin displacer (3) all according to the same dosage schedules as for D penicillamine Blood was drawn from the tail vein before treatment and at intervals thereafter The serum bilirubin concentration was measured according to the method of Mertz & West (18) modified by Jezernicsky (11)

RESULTS

The rate of bilirubin oxidation with peroxidase in a solution of bilirubin/albumin, molar ratio 1/2, with varying concentrations of D penicillamine is depicted in Fig 1 It was found that the drug inhibits the oxidation by a factor 0 50, irrespective of varying drug concentrations from 0 5 to 5 mM In the abscence of albumin, an identical inhibition of the enzyme was observed Oxidation rates, corrected for this inhibition, were constant within the experimental error An unchanged rate of oxidation, with varying concentrations of the drug indicates that the free equilibrium concentration of bilirubin remained constant since only the free bilirubin is oxidized This again demonstrates that D penicillamine in concentrations until 5 mM, does not interfere with binding of bilirubin to albumin under the conditions of this experiment

The MADDS method measures the concentration of available high affinity site for binding of bilirubin (reserve albumin) by determination of the binding equilibrium of a selective ligand, monoacetyl diaminodiphenylsulfone, added in low concentration, 8 μM The concentration of available site is equal to the albumin concentration in a pure solution, since *each albumin molecule has one high affinity* site, and decreases on binding of competitive ligands As seen in Fig 2*a*, the available concentration of binding site remained unchanged with D penicillamine concentrations varying from zero to 5 mM D penicillamine is thus not bound to the bilirubin site on the albumin molecule

Results of a similar experiment with sulfamethizole, which binds competitively to the bilirubin site, and thus may cause displacement of bilirubin *in vivo*, are shown for comparison

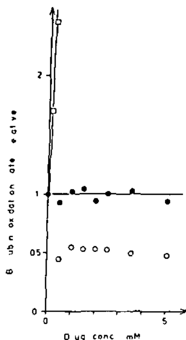


Fig 1 Competitive binding of bilirubin and D penicillamine tested by the peroxidase method Rates of bilirubin oxidation with varying concentrations of D penicillamine O relative to the rate obtained in the absence of the drug are plotted on the ordinate Rates decreased to about one half irrespective of varying D penicillamine concentrations from 0 5 to 5 mM After correction for inhibition of the enzyme also observed in the absence of albumin constant oxidation rates were found ● demonstrating that D penicillamine does not interfere with the binding of bilirubin to human serum albumin Experiments with sulfamethizole a known displacer are shown for comparison □

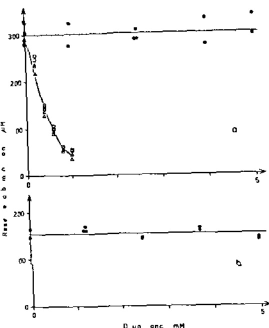


Fig 2 Concentration of available bilirubin binding site *reserve albumin* determined by the MADDS method as a function of varying concentrations of D-penicillamine ● In (*a*) a solution of human serum albumin 300 μM was used and in (*b*) cord blood serum from a healthy infant D-penicillamine does not occupy bilirubin binding sites Experiments using varying concentrations of sulfa methizole □ illustrate competitive binding of this drug Combinations of D-penicillamine 2 5 mM fixed con centration with varying concentrations of sulfamethizole Δ show the same effect as sulfamethizole alone

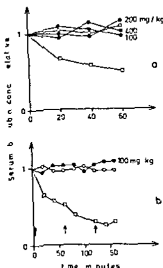


Fig 3 Test for bilirubin displacing effect of D-penicill amine in Gunn rats (*a*) ○ D-penicillamine was injected intravenously in a single dose as indicated in the figure (*b*) ● D-penicillamine 100 mg/kg injected intravenously three times at one hour intervals as indicated by the arro…

…jection and at intervals thereafter Each point represents an average of determinations in 3–5 rats Concentrations relative to the pre treatment level are plotted in the ordi nates D penicillamine does not displace bilirubin in the Gunn rat

The experiment with sulfamethizole was repeated using a fixed concentration of D-penicillamine 2 5 mM and varying sulfon amide concentrations Sulfamethizole (and other sulfonamides) are bound to several sites on the albumin molecule including the high affinity site for bilirubin D penicillamine might bind to albumin at other sites than that for bilirubin and might thus displace sulfa methizole which in turn would compete to an increased extent with the binding of bilirubin An *indirect competition* of D-penicillamine and bilirubin binding might thus result in the presence of a suitable mediator, such as the sulfonamide, even though a *direct competition* for one site does not take place The results, illustrated in Fig 2*a*, show that the compe titive effect of sulfamethizole remains un changed on addition of D penicillamine No in direct competition could be observed

Finally MADDS binding studies were per formed using serum from newborns and vary ing concentrations of D penicillamine with a view to possible indirect competitive effects in the presence of any unknown mediator As seen in Fig 2*b*, no competitive effect was observed

Due to an inherited lack of bilirubin conjuga tion, Gunn rats have a high constant plasma bilirubin concentration The pigment is bound to albumin and is displaced into the tissues after administration of a drug which interferes with bilirubin binding, resulting in a decrease of plasma bilirubin Fig 3 illustrates that D penicillamine caused no displacement while sodium benzoate, as expected, showed a marked effect

DISCUSSION

The clinical significance of bilirubin displacement by drugs was discovered more than twenty years ago through the work of Silverman et al (22) who described an outbreak of kernicterus with a considerable mortality in a group of jaundiced neonates treated with sulfisoxazole The displacement mechanism was established by Odell (20), and Stern in 1972 pointed out that all drugs used in neonatology should be tested for bilirubin displacing effect (24)

The choice of methods for such testing is important and has recently been discussed in detail (6) Several authors have used a Sephadex column technique A solution of bilirubin and albumin, or icteric serum, is passed through the column If addition of the drug causes yellow staining of the Sephadex, the drug is reported as displacing The sensitivity of this method has not been established, its specificity too is questionable, since photobilirubin formed on illumination of the solution, or bilirubin isomers present in the serum, may be taken up by Sephadex and moreover, the method does not give quantitative results

A combination of three techniques was utilized in the present study 1) The peroxidase method is convenient for screening purposes (6) A result indicating abscence of displacing effect, or rather indicating that quantitatively the displacement is less than a certain accepted level at the maximal drug concentration obtainable in plasma, may generally be accepted as final False positives have been found with phenol, phenothiazines and a few other drugs which accelerate the oxidation of bilirubin, irrespective of binding to albumin (7) 2) The MADDS method does not carry the latter type of error and has the furhter advantage that it can be applied to undiluted serum so that drugs can be tested in actual infant serum This is not possible with the peroxidase method On the other hand, the MADDS technique may give somewhat erroneous values in dealing with a drug having allosteric negative or positive, effect on bilirubin binding, if this differs from its effect on the binding of MADDS The possible practical importance of this has not yet been evaluated 3) Finally the Gunn rat test may give additional information if displacing metabolites of a drug are incurred This test, however, should not be used alone, since binding patterns of bilirubin and drugs to rat albumin may differ from those seen with the human protein

The results obtained for D penicillamine indicate absence of displacing effect in all three tests Quantitatively speaking, both the peroxidase and MADDS methods indicate less than 20% displacement at the highest concentration tested, 5 mM Clinically, intravenous doses of 100 mg/kg are given at 8 h intervals for 3 days Since elimination of the drug is rapid, this gives a theoretical maximum of 2 mM D penicillamine if one dose is distributed in the extracellular space In the Gunn rat, displacement was probably less than 10% at the highest dose level, 400 mg/kg It was further found that indirect displacement in the presence of sulfamethizole does not take place also not in infant serum

Steffen (23) studied the effect of D penicillamine on the level of plasma bilirubin in infant Gunn rats Intravenous injection of the drug was impractical in these small animals No effect was seen after oral, intraperitoneal or subcutaneous administration Purkinje cells were not damaged

Consequently the attenuating effect of D penicillamine on the level of plasma bilirubin in jaundiced infants cannot be due to displacement of bilirubin from binding to albumin Previous findings seem to indicate three possible mechanisms of action of this drug viz increased osmotic resistance of erythrocytes decreased immunological response and changes of bilirubin metabolism involving an increase of conjugating enzyme (UDPGA glucuronyl transferase) and a decrease of bilirubin forming enzyme (α haem oxygenase) (14) Further studies on the mechanism of action of D penicillamine in neonatal hyperbilirubinaemia are in progress

ACKNOWLEDGEMENTS

The technical assistance of Signe Andersen Birthe Lindgaard and Nora Blasius is acknowledged The study was supported by a grant from the Danish MRC

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Submitted June 1 1979
Accepted July ?0 1979

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Acta Pædiatr Scand 69 37–41, 1980

SOMATOMEDIN ACTIVITY AND GROWTH HORMONE SECRETION

I Changes Related to Body Weight in Anorexia Nervosa

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ABSTRACT Rappaport R, Prevot, C and Czernichow, P (Department of Paediatrics Hopital des Enfants Malades, Paris, France) Somatomedin activity and growth hormone secretion I Changes related to body weight in anorexia nervosa Acta Paediatr Scand 69 37, 1980.—Anorexia nervosa in childhood and adolescence, associated with impaired growth can be considered as a form of chronic malnutrition Twelve patients aged 11 to 17 years were investigated In spite of increased GH secretion, plasma somatomedin activities were diminished in 8 cases Plasma T_4, T_3 and arginine stimulated insulin secretion were also decreased In six patients who gained weight a significant negative correlation was found between weight deficit and plasma somatomedin activity Prolonged administration of growth hormone in one case did not stimulate the generation of somatomedin activity Nutrition and increased insulin secretion could play a role in changes observed during recovery

KEY WORDS Anorexia nervosa, somatomedin activity, nutrition

Recently it has become apparent that circulating somatomedin levels can be dependent upon nutritional status This was shown in experimental protocols of fasted or diabetic rats (15 16) and in clinical studies of children with malnutrition (10, 11 21) Growth failure in these children was attributed to the deficit in somatomedin generation Anorexia nervosa during childhood or adolescence can be associated with impaired growth (7, 12, 19) and may be considered as a form of chronic malnutrition We studied the relationship between plasma levels of GH and somatomedin activity in anorexia nervosa and determined the effect of weight changes on the generation of SM activity

MATERIAL AND METHODS

Patients The characteristics of the subjects studied are presented in Table 1 The [illegible]

dence of diminished growth rate at the time of study by [illegible]

patients except one were severely underweight at the onset of study between 16% and 41% below the mean ideal weight for height Patients of pubertal age presented a lack of puberty or an abnormal pubertal development with absence of progression of sexual hair and breast development primary or secondary amenorrhea Hypothalamic pituitary lesions were ruled out by pneumoencephalogram when necessary These clinical data were reported in detail elsewhere (7) The duration of the follow up varied from 1 month to 2 years and 8 months In the case DI who had a complete arrest of growth since five years with a bone age of 9 years studies were performed to evaluate the responsiveness to administration of human growth hormone (kindly provided by the French Pituitary Agency) and to administration of l triiodothyronine Except for the child JA who initially required continuous enteral infusion all had free access to food during the study They were not forced to eat and hence weight gains were variable and frequently limited to periods of several months

Most patients refused to be hospitalized in the metabolic ward and it was not possible to evaluate precisely their caloric intake Plasma total protein and albumin concentrations were always in the normal range In six pa

Supported by a grant 42 76-74 of the INSERM France

Table 3 *Changes in GH and insulin secretion and plasma somatomedin activity in six patients before and after weight gain*

M ± S E M and range—Comparison by paired *t* test IBW=ideal body weight for height AITT=arginine insulin tolerance test

Clinical status weight deficit % IBW	GH ng/ml AITT peak	Sm Activity (U/ml)	Insulin μU/ml ARG peak
Control (*n*=6) 19–41%	31±18 (6 1–120 5)	0 24±0 02 (0 20–0 34)	19 8±4 5 (5–33)
After weight gain (*n* 6) 0–22%	7 3±1 4 (3 1–11 8) NS	0 45±0 11 (0 35–1 27) *p*<0 01	33 7±2 8 (26–44) *p*<0 01

test serum was expressed in percentage of the potency of normal plasma at 20% concentration Student's *t* test and paired *t* test and least squares linear regression analysis were used for statistical evaluation

RESULTS

Initial endocrine studies The provoked GH secretion peak values were in the hypopituitary range (below 5 ng/ml) in 2 cases out of 12 Plasma somatomedin activity was diminished in one of these cases and in 9 additional cases where the GH secretion was normal Seven patients with low plasma SM activities presented with a decreased growth rate according to their bone age at the time of initial evaluation (Table 1) The mean plasma GH peak was increased (*p*<0 01) However this is due to two patients (RO and JA) with increased basal and peak GH concentrations All of the other GH values were well within the normal range

Table 4 *Low growth hormone response to arginine insulin stimulation with normal somatomedin activity in patients with anorexia nervosa during weight gain*

AITT=arginine insulin tolerance test

Case	Weight deficit (% IBW)	GH ng/ml AITT peak	Sm Activity (U/ml)	Insulin μU/ml ARG peak
CO	16	1 0	0 73	13
	15	3 5	1 27	35
HU	11	3 2	1 60	25
JU	22	3 1	0/60	26

There was a significant decrease of the mean plasma Sm activity (*p*<0 001), plasma T_3 (*p*<0 001), and T_4 (*p*<0 001), and decrease of the mean arginine stimulated insulin peak (*p*<0 001) The mean blood glucose level was normal There was no significant correlation between plasma insulin peak, T_4 or T_3, glucose and Sm activity (Table 2) Only patient JA who had the most severe weight deficit, was found to present a circulating inhibitory activity during the early period associated with high circulating GH values The potency of a normal plasma was reduced by the addition of the patient's plasma, respectively to 16 82 and 50% of its control value on days 1, 5 and 8 during refeeding by a constant enteral infusion

Relationship between weight gain and plasma Sm activity Six patients gained weight during follow up VA, RO, CO, HU, JA, JU with a weight increase of respectively 9, 6, 6, 10, 14 and 4 kg Each patient had several plasma Sm activity and provoked GH secretion evaluations during this period In order to investigate a possible relationship between changes in weight and circulating plasma Sm activity in this group all individual values were recorded and combined The weight status was expressed as a deficit for ideal body weight for age All patients, except one, remained with a deficit greater than 13% A significantly negative correlation was found between weight deficit and plasma Sm activity (*p*<0 01, *r*=0 62)

Table 1. *Clinical data at time of the initial study of patients with anorexia nervosa*

Ideal BW ideal body weight for height. Pubertal stages according to Tanner (menstruations were absent in all fem patients). Bone age, according to the Atlas of Greulich and Pyle

Bone age (y)	Patient	Growth rate (cm/y)	Age (y)	Height (cm)	Pubertal stage	Weight (kg) (%) below ideal BW
9	DI	0	11	136	No puberty	19 2 (36)
11	VA	2	12 6/12	140	$P_3 A_1 B_2$	21 0 (36)
13	RO	3 5	12 7/12	157	$P_2 A_1 B_2$	29 5 (36)
9	LE	1 5	12 7/12	134 6	No puberty	23 2 (20)
13	TR	1 5	13	153 5	$P_4 A_1 B_2$	29 1 (32)
13	VI	–	14 3/12	154	$P_2 A_1$	34 3 (20)
13	CO	1	15 2/12	156	$P_2 A_2 B_2$	37 5 (16)
15	HU	–	15 3/12	166 5	$P_3 A_2 B_4$	42 5 (19)
13	JA	1	15 6/12	161	$P_4 A_1 B_4$	29 0 (41)
13	JU	0	15 11/12	159	$P_3 A_1 B_3$	30 6 (35)
14	BO	–	15 11/12	142	$P_4 A_3 B_4$	32 5 (4)
17	BA	–	17 7/12	162	$P_3 A_3 B_4$	40 2 (20)

tients with a variable weight gain during the study, repeated evaluations were performed (see below)

Methods Hormonal measurements for each patient were performed on the same day. This included early morning baseline samples for plasma T_3, thyroxine and somatomedin activity, followed by a sequential arginine insulin stimulation test (22). Plasma insulin was measured during the arginine stimulation. Heparinized glass tubes were used and stored at −20°C until assayed. Blood glucose was estimated by the Auto Analyser ferricyanide method. Plasma growth hormone and insulin concentrations were determined by double antibody radioimmunoassay. Plasma T_4 was measured by a competitive ligand assay and T_3 by radioimmunoassay (Amersham Lab Ki Somatomedin (Sm) bioactivity was estimated in plasm samples by the increase in SO_4 incorporation in porci costal cartilage according to Van den Brande (2 0 2 U/ml was the lowest value of Sm detectable procedure for computer assisted parallel line bioass analysis was used. λ values were below 0 20. All valu were compared to a normal adult plasma pool, one ml which defined a unit of Sm activity. Somatomedin inhib tory activity was estimated by the ability of unknow plasma to decrease cartilage stimulation by norm standard plasma in mixing experiments. The somat medin potency of 20% normal plasma with 20% add

Table 2. *Initial studies. Growth hormone and insulin secretion, plasma thyroxine, triiodi thyronine and somatomedin activity*

Control children were prepubertal (6–12 years) for GH, INS, T_4 and T_3. M±S E M. AITT = arginine insulin stimulati test, ARG = arginine

Patient	GH ng/ml Basal	GH ng/ml AITT peak	Sm (U/ml)	T_4 (μg/dl)	T_3 (ng/dl)	Insulin μU/ml ARG peak
DI	1 3	22	<0 2	3 75	0 9	–
VA	1 2	26	<0 2	5 9	0 6	13 2
RO	20	120	<0 2	2 9	0 4	5
LE	1 4	4 2	<0 2	6 5	2 0	40
TR	1 8	30 5	<0 2	4 4	1 1	10 8
VI	0 8	14 2	0 36	5 6	0 9	–
CO	0 9	1 0	0 73	3 8	0 8	13 1
HU	1 3	26	<0 2	4 35	0 5	37
JA	20	74 5	<0 2	1 25	0 2	11
JU	2 4	9 2	<0 2	3 75	0 45	22
BO	<0 75	27	<0 2	7 55	0 95	16 2
BA	2 2	6 7	1 36	4 0	0 9	24 2
Mean	4 5±2 1	30 1±3 0 $p<0$ 01	0 35±0 10 $p<0$ 001	4 5±0 5 $p<0$ 001	0 8±0 10 $p<0$ 001	19 3±3 7 $p<0$ 001
Controls	3 1±0 3	20±1 7 ($n=19$)	0±0 14 ($n=14$)	9±2 2 ($n=12$)	1 8±0 5 ($n=12$)	48 4±4 7 ($n=16$)

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	15	3 5	1 27	35
HU	11	3 2	1 60	25
JU	22	3 1	0/60	26

There was a significant decrease of the mean plasma Sm activity ($p<0\ 001$) plasma T_3 ($p<0\ 001$), and T_4 ($p<0\ 001$), and decrease of the mean arginine stimulated insulin peak ($p<0\ 001$) The mean blood glucose level was normal There was no significant correlation between plasma insulin peak, T_4 or T_3, glucose and Sm activity (Table 2) Only patient JA who had the most severe weight deficit, was found to present a circulating inhibitory activity during the early period associated with high circulating GH values The potency of a normal plasma was reduced by the addition of the patient s plasma, respectively to 16, 82 and 50% of its control value on days 1, 5 and 8 during refeeding by a constant enteral infusion

Relationship between weight gain and plasma Sm activity Six patients gained weight during follow up VA, RO, CO, HU, JA, JU with a weight increase of respectively 9, 6, 6, 10, 14 and 4 kg Each patient had several plasma Sm activity and provoked GH secretion evaluations during this period In order to investigate a possible relationship between changes in weight and circulating plasma Sm activity in this group, all individual values were recorded and combined The weight status was expressed as a deficit for ideal body weight for age All patients, except one, remained with a deficit greater than 13% A significantly negative correlation was found between weight deficit and plasma Sm activity ($p<0\ 01$, $r=0\ 62$)

The mean values before and at time of the maximum weight were respectively 0 24±0 02 and 0 45±0 11 U/ml ($p<0\ 01$) for Sm activity and 19 8±4 5 and 33 7±2 8 μU/ml ($p<0\ 01$) for plasma insulin (paired *t*-test), however, *they remained significantly below the normal* control values (Table 3) Plasma GH peak values when initially elevated, decreased to normal levels (not shown) Four patients achieved a Sm activity in the normal range, above 0 50 U/ml (VA, CO, HU and JU, not shown)

Inability to generate somatomedin activity In addition to the resistance to endogenously secreted growth hormone, the response to exogenously administered human growth hormone was evaluated in patient DJ who was treated with human growth hormone, 12 units/ weekly for five months The plasma Sm activity measured 24 hours after GH injections, on the fourth week (3 times) and twentieth week (3 times) never exceeded 0 30 U/ml (blood was drawn the day after GH injection) During this period she severely restricted her food intake and there was no change of weight or height Later, she received l-triiodothyronine (100 μg/day t i d) for four days and plasma Sm activity remained below normal (0 36 U/ml initially, and 0 48 U/ml after l-T_3)

Low GH response to stimulation and normal Sm activity As shown in Table 4, in three patients after weight gain plasma Sm activities were found in the normal range in spite of inadequate responsiveness of GH to the AITT stimulation Arginine stimulated insulin peak concentrations were in the normal range (48 4±18 8 (S D) μU/ml) None of these patients had reached a normal body weight for their height Patient CO grew as she gained 6 kg In all three cases plasma T_3 and T_4 concentrations were normal

DISCUSSION

The purpose of the present report was to evaluate certain hormonal changes in relation to the severity of weight loss in anorexia nervosa At the time of the initial study of the patients, a pattern of hormonal changes characteristic of malnutrition, was observed increased GH secretion, decreased insulin response and decreased plasma T_3 and T_4

Provoked GH secretion was found to be normal or elevated in most cases as reported by several investigators (9, 13, 18 22) GH deficiency has also been observed (3, 12) and was present in only two of our patients A normal GH rise during slow-wave sleep has been reported (8) The severity of weight loss is significantly correlated with the basal GH levels (22) These data are similar to those found in chronic malnutrition (2), and it is likely that the variable pattern of GH secretion observed in anorexia nervosa, is related to ill defined nutritional conditions

In contrast, plasma somatomedin activity, as measured by the porcine costal cartilage assay was significantly diminished The inability for endogenous and exogenous GH to stimulate somatomedin activity is probably a characteristic feature of undernutrition This is only partly related to the presence of inhibitors in the serum A number of experimental studies showed that fasted rats had a decrease in serum activity (5) and their serum contained inhibitors of the activity of somatomedin in normal serum (17) In malnourished children (10) this has not been a constant finding, and dose response curves were not always parallel to the standard serum curve suggesting that different components contributed to the plasma Sm activity The present study demonstrates a correlation of plasma Somatomedin activity with the weight gain which may reflect an improvement of the nutritional status

It is likely that other factors play a role in Sm activity generation as insulin and thyroid hormones Plasma triiodothyronine and thyroxine concentrations were decreased at the time of the initial evaluation and reached normal values in the group of patients who gained weight, but this was not related to a parallel increase of plasma Sm activity It is

therefore difficult to assess the role of hypothyroidism in our patients, although it was shown that thyroid hormones stimulate sulfate uptake by cartilage in vitro (1) and that Sm activity is decreased in hypothyroidism (21) Changes in insulin secretion after the initial hypoinsulinism (18) may well reflect the increase of caloric intake and stimulate the circulating Sm activity Similar changes have been reported in children with GH deficiency and normal insulin secretion after removal of craniopharyngioma (4), and experimental data support such a role for insulin (6, 16) At physiological concentration the role of insulin on Sm generation remains ill defined In conclusion the diminution of circulating Sm activity appears to be a characteristic feature of the nutritional condition in anorexia nervosa and does not follow the observed changes in provoked GH secretion

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Submitted Febr 7 1979
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Acta Pædiatr Scand 69 43–47, 1980

SERUM LEVELS OF THYROTROPIN, THYROXINE, 3,3′,5-TRIIODOTHYRONINE AND 3,3′,5′-TRIIODOTHYRONINE (REVERSE T_3) IN THE FIRST SIX DAYS OF LIFE

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ABSTRACT Cavallo, L , Margiotta, W., Kernkamp, C and Pugliese, G (Istituto di Puericultura, Università di Bari and CNR, Italy) Serum levels of thyrotropin, thyroxine, [illegible]

gestational age, aged 1 to 6 days, delivered vaginally and breast-fed. Serum TSH levels decreased progressively from the 1st to the 4th day, serum T_4 levels increased, with a peak on the 2nd day, and then progressively decreased until the 6th day, serum T_3 levels increased to a maximum value on the 2nd day and then decreased to a minimum on the 5th day, serum rT_3 levels increased during the 1st day and the level remained constant from the 2nd to the 4th day and later decreased slightly The decrease of T_3 was more pronounced than that of T_4, while rT_3 remained at high levels until the 4th day Dividing the data into narrower intervals of time, it was possible to show that the maximum value of TSH was followed first by a net increase in serum T_3, then in T_4, and lastly in rT_3 ant T_3 levels These data indicate that the rapid increase after birth of serum T_3 levels is prevalently TSH-dependent, the following increase in serum levels of T_3 and the increase in rT_3 are prevalently T_4-dependent. This study provides data concerning physiological changes in TSH and thyroid hormones in serum from a large number of infants, during the first week of life They should be useful for the understanding of thyroid function in early postnatal life

KEY WORDS Thyrotropin, thyroid hormones, thyroxine, 3,3′,5-triiodothyronine, 3,3′,5′-triiodothyronine, postnatal development of thyroid function, full term newborns

In the first hours of postnatal life a rapid and net increase in serum levels of thyrotropin (TSH) has been reported (13, 14, 16, 18, 19, 22, 23), this is followed by a rise in serum levels of 3 3′,5-triiodothyronine (T_3) (1 5, 10, 17 18, 22, 23) and of thyroxine (T_4) (1, 5, 10, 18, 23) But there is no agreement on the behaviour of serum levels of these hormones during the first week of extrauterine life, this may be because of both the small number and the poor characterization of the patients and the heterogeneity of the methods used Moreover, only limited and contradictory information is available about serum levels of 3,3′,5′-triiodothyronine (rT_3) an increase (4), a decrease (3) or no change (7) during the first days of postnatal life has been observed

The present study was carried out to determine serum concentrations of TSH and of thyroid hormones (T_4, T_3, and rT_3) during the first six days of postnatal life in a large and homo geneous population of infants

MATERIAL AND METHODS

A total number of 140 full term newborns with gesta tional ages from 38 to 42 weeks with ages ranging from 1 to 6 days delivered vaginally and breast fed were studied The [illegible]
group consiste [illegible]
tional age grou [illegible]
the subjects were in good clinical condition without in fections, respiratory difficulties fetal distress or hypothermia and they did not come from endemic goiter re

[1] From CNR Italy

Table 3 *Serum T_3 levels (nmol/l) during the first six days of postnatal life*

Conversion *S I* to traditional units *1 nmol/l 65 10 ng/100 ml*

Postnatal age (days)	No of subjects	Postnatal age (h) Median and range	Median	Range	$p\leq$
1	24	9 (1 24)	2 13	0 84–3 63	0 02
2	9	37 (28–48)	3 16	1 95–4 07	0 02
3	16	61 (49–69)	2 14	0 81–3 39	
4	3	76 (75–95)	1 65	0 65–1 90	
5	3	103 (101–113)	0 84	0 84–1 42	
6	5	126 (121–129)	1 50	0 96–1 94	
Controls	17		2 57	1 64–3 11	

The childhood values are reached at about the 60th hour of life (5), but our data do not confirm that they persist at high levels for a long time (16 18) or reach adult values by the 12th hour (23) A statistically significant decrease ($p<0\,02$ and 0 01) in TSH levels was found between the 12th and the 72nd hour of life, when the data were grouped according to intervals of 12 hours

Serum levels of T_4 We have found a net increase in serum levels of T_4 with maximum value on the 2nd day of life, followed by a decrease that becomes progressively less steep Childhood values are reached during the 6th day of life (Table 2) A statistically significant increase ($p<0\,02$) was found between the 12–18 hour and 18–24 hour values and between the 12–24 hour and the 24–36 hour values Previous reports have said there is a significant increase within the first four hours of life (1 23), with high levels until the 48th hour (1, 5, 10, 18, 23) or no modifications (22) Adult values are said not to be reached until the 5th day (10, 18)

Serum levels of T_3 We have found a net increase, with a maximum value on the 2nd day followed by a progressive decline to a minimum on the 5th day with a subsequent elevation (Table 3) A statistically significant increase was found between the values at 0–6 hours and those at 6–12 hours ($p<0\,02$) and between 0–12 hours and 12–24 hours, and 12–24 hours in contrast to 24–36 hour values ($p<0\,01$) and a statistically significant decrease ($p<0\,01$) between the 48–54 hour and 54–60 hour, the 54–60 hour and 60–66 hour values and the 48–60 and 60–72 hour values Serum levels of T_3 have been reported to increase within four hours of life (1, 10 22, 23) and its maximum value to be achieved at the 2nd hour (25) or at the end of the first day (1, 10 18) or on the 2nd day (5) Adult values

Table 4 *Serum rT_3 levels (nmol/l) during the first six days of postnatal life*

Conversion *S I* to traditional units 1 nmol/l 65 10 ng/100 ml

Postnatal age (days)	No of subjects	Postnatal age (h) Median and range	Median	Range	$p\leq$
1	32	10 (1–24)	1 85	1 27–2 98	
2	17	34 (26–42)	2 53	1 63–3 15	0 02
3	16	60 (50–72)	2 46	1 52–3 29	
4	12	82 (73–95)	2 61	1 64–3 21	
5	10	109 (97–120)	1 92	1 34–2 78	
6	8	127 (121–130)	1 73	1 56–2 55	
Controls	20		0 27	0 14–0 38	

Table 1. *Serum TSH levels (mU/l) during the first six days of postnatal life*
Conversion S I to traditional units 1 mU/l = 1 μg/ml

Postnatal age (days)	No of subjects	Postnatal age (h) Median and range	Median	Range	$p \leq$
1	17	15 (8–24)	24 3	11 6–35 9	
2	22	32 (25–42)	11 5	8 3–19 8	0 02
3	13	54 (49–71)	5 9	1 0–10 9	0 02
4–6	13	108 (74–144)	1 5	1 2– 5 8	0 02
Controls	*11*		2 2	1 5– 4 2	

gions, all infants took their first feeding 12 hours after delivery

LGA newborns were included in this research because they did not show any statistically significant difference in serum levels of TSH and of thyroid hormones from AGA newborns (6) The informed consent of the parents was obtained Sera, obtained by venipuncture, sedimentation and centrifugation, were frozen at −20°C until assay Serum concentrations of TSH, T_4, T_3, and rT_3 were measured by radioimmunoassay techniques (Biodata, Milano) and all measurements were made in triplicate Two to four variables were measured in each blood sample The control serum was a pool of normal adult sera (Biodata, Milano) Normal values were obtained from healthy children with constitutional short stature, 3–12 years old

The control and unknown serum concentrations were measured in the following dilutions 1 2 and 1 4 (TSH and T_3), 1 20 and 1 40 (T_4), 1 1 and 1 2 (rT_3) Mean values and standard deviations of control sera were 3 73±0 70 mU/l (TSH), 77 99±6 95 nmol/l (T_4), 1 36±0 09 nmol/l (T_3), 0 22±0 02 nmol/l (rT_3) Within assay coeffi- [illegible]

(rT_3) Non-specific bindings (NSB) were 2 63±1 77% (1 1–5 9%) for TSH, 4 40±0 40% (4 0–4 9%) for T_4 4 29±1 67% (2 4–5 6%) for T_3, 2 57±0 70% (1 6–3 5%) for rT_3 Total bindings were 36 55±6 10% (27 0–43 7%) for TSH 34 21±3 25% (29 5–38 5%) for T_4, 36 81±4 54% (33 5–44 0%) for T_3, 38 18±5 94% (33 6–47 0%) for rT_3 There was no cross-reaction of TSH antiserum with up to 2 500 mU/ml (2nd I R P -hMG) of FSH, up to 35 000 mU/ml (2nd I S -hCG) of hCG, up to 5 000 μU/ml (W H O 66/217) of hGH and up to 5 000 ng/ml (N I H -hPRL-F1) of hPRL Cross-reaction of T_4 antiserum was 100% with L-T_4, 0 37% with L-T_3, 1 02% with rT_3 Cross-reaction of T_3 antiserum was 100% with L-T_3, 80% with D-T_3, 0 02% with L-T_4, less than 0 005% with D-T_4 Cross reaction of rT_3 antiserum was 100% with rT_3, 0 1% with L-T_4, 0 004% with L-T_3

Statistical analysis The data obtained were grouped according to the days of life and to intervals of 12 and 6 hours The Mann-Whitney bidirectional non-parametric statistical test for unpaired data was used for the evaluation of the null hypothesis (no true differences between the groups) and the Wilcoxon test (2) The null hypothesis was rejected at the 0 05 level of significance

RESULTS AND DISCUSSION

Median values and range for serum TSH, T_4, T_3, and rT_3 levels during the first six days of postnatal life, expressed as Standard International (S I.) units, are summarized in Tables 1–4

Serum levels of TSH. Our data confirm that serum TSH levels (Table 1) show a net increase during the first hours of postnatal life (13, 14, 16, 18, 19, 22, 23), followed by a slow decline during the first two days (5, 13)

Table 2. *Serum T_4 levels (nmol/l) during the first six days of postnatal life*
Conversion S I to traditional units 1 nmol/l=0 078 μg/100 ml

Postnatal age (days)	No of subjects	Postnatal age (h) Median and range	Median	Range	$p \leq$
1	24	12 (3–24)	170 5	99 1–275 4	0 02
2	21	35 (25–48)	223 9	155 7–317 9	
3	24	60 (49–72)	211 7	135 1–251 0	0 02
4	12	82 (73–95)	163 5	135 1–225 2	
5	10	109 (101–120)	161 5	122 3–231 7	
6	3	130 (123–143)	141 6	113 3–175 0	
Controls	14		106 8	61 8–144 1	

Table 3 *Serum T_3 levels (nmol/l) during the first six days of postnatal life*

Conversion S I to traditional units 1 nmol/l 65 10 ng/100 ml

Postnatal age (days)	No of subjects	Postnatal age (h) Median and range	Median	Range	p≤
1	24	9 (1–24)	2 13	0 84–3 63	0 02
2	9	37 (28–48)	3 16	1 95–4 07	0 02
3	16	61 (49–69)	2 14	0 81–3 39	
4	3	76 (75–95)	1 65	0 65–1 90	
5	3	103 (101–113)	0 84	0 84–1 42	
6	5	126 (121–129)	1 50	0 96–1 94	
Controls	17		2 57	1 64–3 11	

The childhood values are reached at about the 60th hour of life (5) but our data do not confirm that they persist at high levels for a long time (16 18) or reach adult values by the 12th hour (23) A statistically significant decrease ($p<0.02$ and 0 01) in TSH levels was found between the 12th and the 72nd hour of life, when the data were grouped according to intervals of 12 hours

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Table 4 *Serum rT_3 levels (nmol/l) during the first six days of postnatal life*

Conversion S I to traditional units 1 nmol/l=65 10 ng/100 ml

Postnatal age (days)	No of subjects	Postnatal age (h) Median and range	Median	Range	p≤
1	32	10 (1–24)	1 85	1 27 2 98	
2	17	34 (26–47)	2 53	1 63–3 15	0 02
3	16	60 (50–72)	2 46	1 52–3 29	
4	12	82 (73–95)	2 61	1 64–3 21	
5	10	109 (97–120)	1 92	1 34–2 78	
6	8	127 (121 130)	1 73	1 56–2 55	
Controls	20		0 27	0 14–0 38	

Table 1. *Serum TSH levels (mU/l) during the first six days of postnatal life*
Conversion S I to traditional units 1 mU/l = 1 μg/ml

Postnatal age (days)	No of subjects	Postnatal age (h) Median and range	Median	Range	$p\leq$
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2	22	32 (25–42)	11 5	8 3–19 8	0 02
3	13	54 (49–71)	5 9	1 0–10 9	0 02
4–6	13	108 (74–144)	1 5	1 2– 5 8	0 02
Controls	11		2 2	1 5– 4 2	

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LGA newborns were included in this research because they did not show any statistically significant difference in serum levels of TSH and of thyroid hormones from AGA newborns (6) The informed consent of the parents was obtained Sera, obtained by venipuncture, sedimentation and centrifugation, were frozen at −20°C until assay Serum concentrations of TSH, T_4 T_3, and rT_3 were measured by radioimmunoassay techniques (Biodata, Milano) and all measurements were made in triplicate Two to four variables were measured in each blood sample The control serum was a pool of normal adult sera (Biodata, Milano) Normal values were obtained from healthy children with constitutional short stature, 3–12 years old

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[illegible] (1 1–5 9%) for TSH, 4 40±0 40% (4 0–4 9%) for T_4 4 29± 1 67% (2 4–5 6%) for T_3, 2 57±0 70% (1 6–3 5%) for rT_3 [illegible] 26 55±6 10% (27 0–43 7%) for TSH [illegible] 36 81±4 54% (33 5– [illegible] 0%) for rT_3 There was no cross-reaction of TSH antiserum with up to 2500 mU/ml (2nd I R P hMG) of FSH, up to 35 000 mU/ml (2nd I S -hCG) of hCG, up to 5000 μU/ml (W H O 66/217) of hGH and up to 5000 ng/ml (N I H -hPRL-FI) of hPRL Cross-reaction of T_4 antiserum was 100% with L-T_4, 0 37% with L-T_3, 1 02% with rT_3 Cross reaction of T_3 antiserum was 100% with L-T_3, 80% with D-T_3 0 02% with L-T_4, less than 0 005% with D-T_4 Cross reaction of rT_3 antiserum was 100% with rT_3, 0 1% with L-T_4 0 004% with L-T_3

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RESULTS AND DISCUSSION

Median values and range for serum TSH, T_4, T_3, and rT_3 levels during the first six days of postnatal life, expressed as Standard International (S.I) units, are summarized in Tables 1–4.

Serum levels of TSH Our data confirm that serum TSH levels (Table 1) show a net increase during the first hours of postnatal life (13, 14, 16, 18, 19, 22, 23), followed by a slow decline during the first two days (5, 13)

Table 2. *Serum T_4 levels (nmol/l) during the first six days of postnatal life*
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3	24	60 (49–72)	211 7	135 1–251 0	
4	12	82 (73–95)	163 5	135 1–225 2	0 02
5	10	109 (101–120)	161 5	122 3–231 7	
6	3	130 (123–143)	141 6	113 3–175 0	
Controls	14		106 8	61 8–144 1	

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Submitted July 3 1978
Accepted July 24 1979

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have been reported to be reached within the 1st day of life (17) or later (1, 5, 10, 18)

Serum levels of rT_3 We have found a net increase between the 1st and the 2nd day with persisting high levels during the 2nd, 3rd, and the 4th day, followed by a slight decline They remain much higher than childhood values during the 6th day (Table 4) A statistically significant increase ($p<0.02$) was found between the 12–24 hour and 24–36 hour values The previous studies on the serum levels of rT_3 in newborns are not in agreement with our results Serum rT_3 is reported to be unchanged (7) or decreased (3) during the first two days of life

In conclusion, our data indicate that after delivery there is a net, early increase in serum levels of TSH followed by a progressive decrease This is followed by the first increase in serum T_3 levels and later by the increase in T_4, T_3, and rT_3 serum levels The first rapid increase in T_3 is precocious and it seems to be due to TSH-stimulation, prevalently, while T_4 and rT_3 do not show corresponding increases The second increase in T_3 and the increase in rT_3 are at the end of the first day of life and they follow the increase in T_4 They seem to be due prevalently to T_4 deiodination The present study seems to confirm that rT_3 is derived mainly from the monodeiodination of T_4 (8, 9, 15)

It is possible to exclude the possibility that these variations in serum thyroidal hormones are the result of different amounts of binding to the thyroid binding globulins (TBG) because TBG levels remain practically unchanged during the first days of postnatal life (10, 19, 21)

The heterogeneity of the data previously reported in the literature may be due to many factors the known effect of environmental temperature on thyroid activity in the newborn (11, 12, 13), that is either unspecified in the report or heterogeneous (10, 13 16, 18), the differences among the modes of feeding of the newborns studied (24) generally unspecified, the differences between the time in postnatal life when studied and the grouping of the data plus the fact that some studies were carried out longitudinally and some transversely

For these reasons, the thyroid parameters estimated by us in a large number of homogeneously selected infants could be used as reference standards for serum TSH, T_4 T_3 and rT_3 levels during the first week of post natal life

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PUBERTAL GROWTH AS REFLECTED BY SIMULTANEOUS CHANGES IN BONE MINERAL CONTENT AND SERUM ALKALINE PHOSPHATASE

S KRABBE, C CHRISTIANSEN, P RODBRO and I TRANSBØL

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reflected by simultaneous changes in bone mineral content and serum alkaline phosphatase Acta Paediatr Scand, 69 49, 1980.—Bone mineral content and total serum alkaline phosphatase were measured simultaneously in 230 normal children and adolescents aged 7–20 years The bone mineral content showed almost no variation from the age of 7 to 13 in boys

correlation between bone mineral content and serum alkaline phosphatase was seen both in boys ($r=0.71, p<0.001$) and in girls (r 0.79, $p<0.001$)

KEY WORDS Bone mineral content, serum alkaline phosphatase, adeolescence

Skeletal growth is an essential feature of physical development The measurement of bone mineral content (BMC) by photon absorptiometry (2) is now established as a method of examining skeletal status

Serum alkaline phosphatase also reflect osteoblastic activity The variation of serum alkaline phosphatase and of height velocity in normal children has been shown to be similar (4) In a more recent report a moderate correlation between increase of height and serum alkaline phosphatase was found in normal schoolboys (5) Mazess & Cameron (10) found that BMC was well correlated to height and weight between ages 7 and 14 years Both parameters exhibit great changes especially during puberty (1, 3, 4, 11) An association between BMC and serum alkaline phosphatase during the years of rapid growth could therefore be expected. We have measured these parameters in normal children and adolescents

SUBJECTS AND METHODS

230 normal children and adolescents aged between 7 and 20 years participated They were randomly selected am

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Acta Pædiatr Scand 69 49–52, 1980

PUBERTAL GROWTH AS REFLECTED BY SIMULTANEOUS CHANGES IN BONE MINERAL CONTENT AND SERUM ALKALINE PHOSPHATASE

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ABSTRACT Krabbe, S , Christiansen, C , Rødbro, P and Transbøl, I (University Clinic of Paediatrics, Children's Hospital Fuglebakken, Copenhagen F, Department of Clinical Chemistry, Glostrup Hospital, Glostrup, Department of Clinical Physiology Aalborg Sygehus Syd, Aalborg, and Division of Endocrinology, Department of Internal Medicine, Hvidovre Hospital, University Hospital of Copenhagen, Hvidovre, Denmark) Pubertal growth as reflected by stimultaneous changes in bone mineral content and serum alkaline phosphatase Acta Paediatr Scand, 69 49, 1980 —Bone mineral content and total serum alkaline phosphatase were measured simultaneously in 230 normal children and adolescents aged 7–20 years The bone mineral content showed almost no variation from the age of 7 to 13 in boys

tions of total serum alkaline phosphatase increased up to the age of 14 in boys and 11 in girls Thereafter a rapid fall was seen in both sexes, the mean levels being significantly higher in boys The fall approximated adult levels in boys by the age of 20 and in girls by 18 A significant negative correlation between bone mineral content and serum alkaline phosphatase was seen both in boys (r 0 71, $p<0$ 001) and in girls ($r=0$ 79, $p<0$ 001)

KEY WORDS Bone mineral content, serum alkaline phosphatase, adeolescence

Skeletal growth is an essential feature of physical development The measurement of bone mineral content (BMC) by photon absorptiometry (2) is now established as a method of examining skeletal status

Serum alkaline phosphatase also reflect osteoblastic activity The variation of serum alkaline phosphatase and of height velocity in normal children has been shown to be similar (4) In a more recent report a moderate correlation between increase of height and serum alkaline phosphatase was found in normal schoolboys (5) Mazess & Cameron (10) found that BMC was well correlated to height and weight between ages 7 and 14 years Both parameters exhibit great changes especially during puberty (1, 3, 4, 11) An association between BMC and serum alkaline phosphatase during the years of rapid growth could therefore be expected. We have measured these parameters in normal children and adolescents

SUBJECTS AND METHODS

230 normal children and adolescents aged between 7 and 20 years participated They were randomly selected among the pupils of two schools in a surburban area

Written consent was obtained from parents for pupils below 18 years of age All were in good health without symptoms of gastrointestinal or renal diseases None took contraceptive pills or any other type of drug

Bone mineral content was determined by direct photon absorptiometry (2) Total serum alkaline phosphatase levels were measured by a modification of the method of March et al (8) and expressed in S I units The coefficients of variation in duplicate measurement were 2 1%

Table 1 *Mean height (cm) in 230 normal children subdivided according to sex and age*

Age (years)	Boys			Girls		
	n	Mean	S D	*n*	Mean	S D
7– 8	10	130 4	5 5	8	131 0	6 8
9–10	11	142 5	9 0	10	144 2	8 5
11–12	17	147 1	6 3	16	151 6	9 0
13–14	14	165 5	12 3	15	164 0	7 0
15–16	20	174 3	10 3	22	165 5	5 9
17–18	26	180 7	6 4	20	166 3	5 8
19–20	21	177 3	5 1	20	167 4	5 0

The surface area of each individual was calculated from the Du Bois nomogram Standard statistical methods were used for determinations of means and correlation coefficients (6)

RESULTS

The height in relation to age and sex is given in Table 1, and correlation coefficients between BMC and age, height, weight, and surface area are given in Table 2 In the prepubertal age groups, only small correlations were seen in boys and in girls The correlation coefficients did not reach significance at the 5% level After puberty between ages 14 and 20 years in boys and 12–20 years in girls a fairly high association between these parameters was seen A very significant correlation between BMC and the indices of body size for the entire span of years is seen in both sexes (Table 2)

Changes of the concentrations of serum alkaline phosphatase and bone mineral content in the various age groups are shown in Fig 1 (boys) and Fig 2 (girls)

BMC values rose from the 13th and 11th years in boys and girls Before puberty mean values in boys were significantly higher than those in girls and this sex difference was highly significant from the 15th year There was no significant sex difference in the concentrations of alkaline phosphatase from the 7th to the 12th year The mean levels decreased sharply from the 14th year in boys and from the 11th year in girls From the 12th to the 18th year the boys had significantly higher mean values than the girls ($p<0$ 001) Maximum values were seen at age 14 and 11 years in boys and girls respectively

A highly significant negative correlation between BMC and alkaline phosphatase is seen

Table 2 *Relation between bone mineral content (BMC) and clinical data in 230 normal children*

The age groups are separated by the time of maximal increase in height (Table 1)

Age groups	*n*	Clinical data			
		Height	*Weight*	Surface area	*Age*
Boys					
7–13	42	$r=0$ 31 $p<0$ 05	$r=0$ 37 $p<0$ 05	$r=0$ 35 $p<0$ 05	$r=0$ 13 N S
14–20	77	$r=0$ 52 $p<0$ 001	$r=0$ 70 $p<0$ 001	$r=0$ 69 $p<0$ 001	$r=0$ 73 $p<0$ 001
7–20	119	$r=0$ 84 $p<0$ 001	$r=0$ 89 $p<0$ 001	$r=0$ 88 $p<0$ 001	$r=0$ 88 $p<0$ 001
Girls					
7–11	26	$r=0$ 38 N S	$r=0$ 14 N S	$r=0$ 25 N S	$r=0$ 07 N S
12–20	85	$r=0$ 46 $p<0$ 001	$r=0$ 42 $p<0$ 001	$r=0$ 49 $p<0$ 001	$r=0$ 68 $p<0$ 001
7–20	111	$r=0$ 78 $p<0$ 001	$r=0$ 73 $p<0$ 001	$r=0$ 78 $p<0$ 001	$r=0$ 86 $p<0$ 001

N S = not significant ($p>0$ 05)

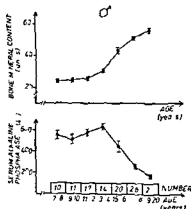


Fig 1 Bone mineral content (BMC) and serum alkaline phosphatase(s) in relation to age in 119 normal boys Values given are mean ± 1 standard error of mean

in Fig 3 (boys $r=-0.72$, $p<0.001$, girls $r=-0.81$, $p<0.001$) This correlation was calculated on all age groups in sum since both parameters exhibited principally similar courses with unchanged values in prepubertal years and rapid changes after the pubertal growth spurt sets in

DISCUSSION

The purpose of this study was to establish the time course of skeletal growth and mineralization as reflected by BMC and total serum alkaline phosphatase between the ages of 7 and 20 years, thus covering physical changes associated with normal puberty. Correlation analyses covering the entire period reveal inter relations between this parameter and height, weight, surface area and age of high statistical significance in both sexes (Table 2) Similar observations were made by Mazess & Cameron (10) in a study of children aged 7 to 14 years However, this simple way of proceeding is not justified from an endocrine point of view Many physiological processes undergo quantitative and even qualitative changes concurrently with the growth spurt which takes place at the age of 11 to 12 years in girls and between 13 to 14 years in boys (Table 1) (9)

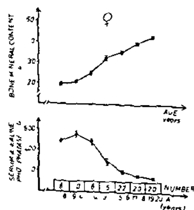


Fig 2 Bone mineral content (BMC) and serum alkaline phosphatase(s) in relation to age in 111 normal girls Values given are mean ± 1 standard error of mean

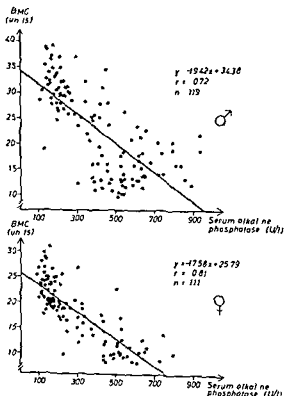


Fig 3 The correlation between bone mineral content (BMC) and serum alkaline phosphatase(s) in 119 normal boys (above) and 111 normal girls (below) aged 7–20 years

Subdivision of our study populations according to this event revealed correlations between BMC and the indices of body size and age of high statistical significance only following the initiation of the growth spurt

The pubertal growth spurt is preceded by a long period of fairly rapid longitudinal growth and coincides with a steep increase in serum testosterone in boys (9) Subsequently, the height velocity decreased very evidently (Table 1) BMC, on the other hand, increases very little ahead of the spurt, but rises steeply afterwards (Figs 1 and 2) Thus as longitudinal growth slows down, appositional bone growth and mineralization accelerates The changes in the levels of serum alkaline phosphatase are also synchronized with the growth spurt high levels are maintained as long as longitudinal growth proceeds rapidly and little mineralization takes place As growth decelerates and the rate of mineralization speeds up as evidenced by BMC serum alkaline phosphatases decrease towards the level of normal adults (Figs 1 and 2) These inverse changes in the levels of alkaline phosphatase and the rates of mineralization appear contradictory since it is generally held that bone alkaline phosphatase, by its virtue of being a pyrophosphatase, facilitates mineralization (7) This apparent contradiction may have its explanation in changing bone cell dynamics Thus rapid bone cell proliferation during rapid longitudinal growth may release large amounts of bone alkaline phosphatase to the systemic circulation Following the growth spurt this contribution of endosteal bone cell turnover may decrease considerably at the same time as gonadal hormones stimulate the conversion of periostal fibroblasts into osteoblasts which presumably contribute greatly to appositional bone growth and mineralization Considering these concomitant changes appositional mineralization can easily proceed despite a net decrease in serum alkaline phosphatase derived from both sources The earlier peak of alkaline phosphatase in girls match with their earlier maturation and growth spurt as observed by others (11) In a recent work (1) the concentrations of total serum alkaline phosphatase were related to sex maturity ratings, and the highest mean concentrations for girls occurred at pubertal stage 2 and for boys at stage 3, in which stages the peak height velocity is attained and just after the rise of gonadal hormones (9) From the time of the peak elevation of serum testosterone in boys serum alkaline phosphatases decrease substantially over the next few years (Fig 1) From a practical point of view our data illustrate that inadequate reference tables are produced if data derived from children of both sexes and all ages in adolescence are included in one single or just a few groups

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Submitted April 2 1979
Accepted July 15 1979

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Acta Pædiatr Scand 69 53–58, 1980

POST-NATAL DEVELOPMENT OF FACTOR IX

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ABSTRACT Schettini F , De Mattia D , Altomare M and Montagna O (University of Bari, Institute of Child Health, Bari, Italy) Postnatal development of factor IX Acta Paediatr Scand, 69 53, 1980 —Post natal development of clotting activity and of antigen level of Factor IX was evaluated in 111 healthy, breastfed, newborn infants, aged 1–30 days Of these, 80 had received at birth 2 mg of vitamin K_1 orally Factor IX clotting activity was determined by one-stage assay and antigen level by electroimmunoassay On the 1st day both antigen level and clotting activity were low and the ratio was 1 01 There was a significant postnatal increase of the two [illegible]

correlation with age The Factor IX protein of newborns did not show molecular heterogeneity by crossed-immunoelectrophoresis

KEY WORDS Factor IX, factor IX antigen, post natal development, newborns, vitamin K_1

Administration of Vitamin K to the newborn has been suggested to prevent a fall of K-dependent clotting factors, to induce normalization of prothrombin time and thromboplastin generation test and to prevent haemorrhagic disease (11 19, 23)

In contrast van Doorm et al (31) reported that the plasma of the newborn does contain protein abnormalities induced by lack of vitamin K and therefore healthy babies, contrary to current belief are not likely to have vitamin K deficiency A heparin-like inhibitor (31) and also a PIVKA-like inhibitor (16) have been reported in the plasma of some newborns

The study of antigen level and of clotting activity of vitamin K dependent factors may contribute to explain the role of vitamin K deficiency in the reduced clotting activity of these factors during the early post natal life

In previous studies (24, 25) we observed that the increase of prothrombin antigen level is progressive after the neonatal period The plasma level reaches the lower limit of the normal range for adults between 4 and 7 months of age and continues to rise slowly through the first year After this age the concentration of the antigen remains constant The clotting activity of prothrombin, on the other hand, reaches the adult range between 15 and 120 days (25) In newborns not treated with vitamin K_1 the clotting activity of prothrombin diminishes significantly on the third day of life and the increase on the fifth day is less marked (24)

In this paper we present the results of our investigation of the post natal development of Factor IX as clotting activity and as antigen level and of the effect of vitamin K_1 administration after birth The purpose of the study was to establish whether the known low level of Factor IX clotting activity (1, 5, 8, 26, 32) in the neonatal period is due to a reduced synthesis of the precursor molecule or to a defect in the biosynthesis of biologically active factor because of vitamin K deficiency

MATERIALS AND METHODS

The clinical material includes 111 healthy infants of weight appropriate for gestational age and breast fed

Table 1. *Weight and gestational age of the infants*
Mean values ± standard deviation

Age group (days)	No.	Weight (g)	Gestational age (weeks)
With administration of Vitamin K_1			
1	16	3 316±0 381	39 5±1 09
3	16	3 528±0 372	38 8±0 80
5	16	3 506±0 403	39 5±1 09
7	17	3 450±0 360	39 1±1 07
10/30	15	3 526±0 410	39 0±1 03
Without administration of Vitamin K_1			
1	10	3 445±0 481	39 1±0 73
3	10	3 380±0 350	39 0±1 05
5	11	3 381±0 321	39 3±1 28

from 1 to 30 days old. The weight and the gestational age (mean value ± standard deviation) are shown in Table 1.

After the birth 2 mg of vitamin K_1 (Konakion Roche) were given orally to 80 infants. The first blood sample was taken within the first day of life. Only one blood sample was obtained from each child and informed consent was obtained from the parents. All infants had a normal clinical course during the time of the research.

Blood obtained by venipuncture with plastic syringes was collected in siliconized glass tubes containing one part of sodium citrate 0.11 M for nine partes of blood (v/v). The blood was centrifuged at 2 200 r.p.m. for 10 min and the plasma was stored at −20°C for not more than 30 days.

Factor IX. One stage assay. The test was performed with an artificial reagent (Hemodiagnostica Stago Reagent Factor IX. Coagulation Diagnostica Boehringer Mannheim) (17).

The clotting times were recorded with an automatic instrument (Clot Timer Mechrolab mod. 202 A Heller Lab. Santa Rosa Calif.) at 37°C.

The plasma was diluted 1:9 (v/v) with imidazole buffer pH 7.4.

The following reagents were placed in the disposable reaction cups:

0.05 ml of citrate plasma dilution (1:9)

0.05 ml of barium sulphate adsorbed normal human plasma

0.05 ml of cephalin

0.05 ml of Kaolin suspension 5 mg/ml (well mixed)

After 3 min of pre incubation at 37°C were added

0.05 ml of avian plasma

0.05 ml of pre warmed 0.025 M $CaCl_2$

The clotting time was recorded in sec and was converted to per cent (%) of activity from a calibration curve prepared with a pool of citrated plasma of healthy adult donors. This mixed plasma was diluted with imidazole buffer pH 7.4 1:10 (100%) 1:20 (50%) 1:40 (25%) 1:100 (10%) 1:200 (5%) and 1:1000 (1%). The mean clotting activity obtained from assay of each dilution in duplicate was plotted on double logarithmic paper and a calibration curve was plotted through the points. The results of One Stage Factor IX assay were expressed as SI (International System) Units (34).

Factor IX. Antigen level assay. The assay was carried out by the electroimmunoassay of Laurell (13) on samples of plasma and in 0.9 g/100 ml agarose gel (100×100×1.5 mm glass plates). The agarose (Behringwerke AG) was dissolved in barbital buffer (TRIS barbital sodium barbital Gelman Instr. Comp.) pH 8.8 and ionic strength 0.05. The anti serum to clotting Factor IX (Clotimmun Factor IX Behringwerke AG Marburg FRG) was obtained by immunizing rabbits with human Factor IX highly purified according to Anderson et al. (2). Unstable serum components, particularly lipoproteins were removed and rabbit clotting factors contained in the anti serum were inactivated. Antibodies against other plasma proteins were removed by absorption with the side fractions obtained during plasma fractionation. The titre (T) indicates how many units of the clotting factor are inactivated by 1.0 ml of anti serum. The anti serum used by us had a titre of 20 units/ml of Factor IX. The specific anti serum gave only one precipitation line in two-dimensional immunoelectrophoresis of plasma. Incubation of the specific anti serum with pooled human plasma leads to a decrease only of the activity of Factor IX but not of the other factors of the so-called prothrombin complex. The anti serum gave no line of precipitation with haemophilia B plasma.

The plasma samples (15 µl) were applied in 12 circular wells of 4.0 mm diameter. Normal pooled plasma undiluted and diluted with 0.15 M saline in the proportion of 1:2, 1:4 and 1:8 (v/v) was tested for each plate. Electrophoresis was carried out for 16 hours at 2.1 V/cm with water cooling (Behringwerke AG).

The reservoir buffer was TRIS Barbital pH 8.8 and of ionic strength 0.05. The gel plates were washed in saline 0.15 M for 24 hours and in distilled water for 60 min followed by 0.025 $CaCl_2$ for 10 min. Finally the plates were dried at 37°C and stained with Coomassie brilliant blue 5 g/100 ml (BDH Chemicals). The destaining was obtained with ethanol 96%–glacial acetic acid–distilled water (450/100/450–v/v/v).

The rocket heights of precipitin lines (Fig. 1) were measured in mm and the values of Factor IX antigen were expressed as percentage of normal pooled plasma. The percentage was obtained from a standard reference curve constructed on semi logarithm paper by testing a series of dilutions prepared from normal pooled plasma.

The sensitivity of the method was not inferior to 0.10 SI, the accuracy was of 3.9%, the precision of between assay was of 5.3% and within assay was of 4.6% (coefficient of variation).

The results of Factor IX antigen were expressed as SI Units (34).

Crossed immunoelectrophoresis of Factor IX was performed according to the modified method (6) of Laurell (15) on 18 µl of normal human plasma pool and on plasma of 5 day old newborns from both vitamin K_1 treated and untreated groups.

For the first dimension run of crossed immunoelectrophoresis 1.5 g/100 ml of Agarose gel (Behringwerke AG) was prepared in buffer TRIS sodium barbital barbital

Table 2 *Factor IX concentration in full term infants*

Determination by one stage assay and by antigen assay during the first month of life (mean values ± standard deviation)

Age group (days)	No	Factor IX* One stage assay	Factor IX* Antigen assay	Ratio Factor IX activity / Factor IX antigen
After administration of Vitamin K₁				
1	16	0 318+0 117	0 313±0 124	1 01
3	16	0 493±0 149	0 552±0 099	0 89
5	16	0 504±0 103	0 506±0 083	0 99
7	17	0 505±0 147	0 468±0 100	1 07
10/30	15	0 406±0 173	0 426±0 113	0 95
Without administration of Vitamin K₁				
1	10	0 305+0 088	0 335±0 164	0 91
3	10	0 517±0 148	0 495±0 204	1 04
5	11	0 504±0 109	0 475±0 089	1 06

* SI Units (Units %×0 01 = SI Units)

(Gellman Instr Comp Ann Arbor) pH 8 8 ionic strength 0 03 The migration was obtained with 10 V/cm for 80 min The immunoelectrophoresis was performed in 1 g/100 ml of Agarose gel (9 0 ml) with Factor IX anti serum (50 µl) at 2 5 V/cm for 18 hours Crossed immunoelectrophoresis was carried out also in presence of Ca^{2+} calcium lactate was added in concentration of 0 0025 M to TRIS-sodium barbital barbital (1.54 g of Ca lactate in 2000 ml of buffer) Human albumin was chosen as a standard protein for mobility comparison

Statistical analysis were performed using an Olivetti minicomputer P6060 mean standard deviation linear regression equation with coefficient of regression (r) Student s t test were obtained

RESULTS

The results (SI Units) are shown in Table 2

Factor IX One-stage assay The clotting activity of Factor IX after administration of vitamin K_1 was on the 1st day 0 318±0 117 and increased to 0 493±0 149 on the 3rd day (p<0 001) It became then constant from the 5th to 30th day (p=NS, r=−0 207) When vitamin K_1 was not administered the value on the 1st day was 0 305±0 088, this rose to 0 517±0 148 on the 3rd day (p<0 01) and remained constant at 0 504±0 104 after the 5th day

Factor IX antigen level The immunochemical determination showed an antigen level at birth of 0 313±0 124 The Factor IX antigen increased to 0 552±0 099 on the 3rd day (p<0 001) After this age the level became



Fig 1 Electroimmunoassay of Factor IX in plasma samples in the following order from the left (*1*) Newborn 3-day-old (no vitamin K) (*2*) newborn 17-day-old (with vitamin K) (*3*) newborn 5-day-old (with vitamin K) (*4*) newborn 1-day-old (no vitamin K) (*5*) pooled adult ... vitamin K)

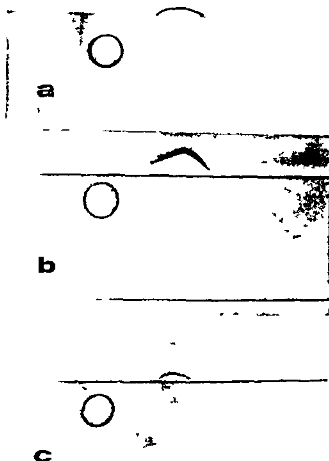


Fig 2 Crossed immunoelectrophoresis of Factor IX (*a*) Newborn 5 day old (after vitamin K_1) (*b*) Adult plasma pool (*c*) Newborn 5-day old (without vitamin K_1)

constant from the 5th to the 30th day (p=NS, $r=-0\ 244$) When vitamin K_1 was not administered the Factor IX antigen was 0 335±0 164 on the 1st day and rose to 0 495±0 204 on the 3rd day of life and to 0 475±0 089 on the 5th day ($p<0\ 05$)

Influence of vitamin K_1 on the development of Factor IX clotting activity Between the two groups of newborns—with and without administration of vitamin K_1—no differences in clotting activity were found (p=NS) in the first five days The rise of the clotting activity was similar in the two groups of newborns and was well correlated on the 3rd day with the increase of antigen level ($r=0\ 653$, $p<0\ 01$ and respectively $r=0\ 807$, $p<0\ 01$) The ratio of clotting activity to antigen level of Factor IX was stable between 0 91 and 1 06

Relationship of Factor IX clotting activity to Factor IX antigen level The Factor IX activity and Factor IX antigen values showed a linear correlation and the coefficient of correlation was 0 761 ($p<0\ 01$) for the group treated with vitamin K_1 and was 0 778 ($p<0\ 01$) for the group not treated

Crossed immunoelectrophoresis The runs showed only one precipitate peak with plasma of newborns whether they had received vitamin K_1 or not Plasma from newborn infants did not differ from that of adults The electrophoretic mobility was also similar for adult and newborn plasma in the presence of CA^{2+} (Fig 2 and 3)

DISCUSSION

The mechanism by which vitamin K controls the synthesis of prothrombin (Factor II) and other vitamin K dependent factors—namely Factors VII, IX and X—has been under active investigations during the last years

Vitamin K is needed for the conversion of a preformed polypeptide precursor into the active coagulation factor (20, 21) The synthesis of pre-prothrombin is not influenced by vitamin K

From studies on bovine prothrombin (7, 9, 18, 22) it is known that vitamin K has a post ribosomal function (20, 29) and is involved in a carboxylation reaction resulting in the formation of gamma carboxyl glutamic acid residues in the N terminal part of the molecule These residues are involved in the binding of calcium and probably of phospholipid as well (27) The prothrombin synthesis may be unrelated to the vitamin K status (20, 28, 29) and may be dependent on the plasma levels of prothrombin itself

Recently an enzyme—called Factor II synthetase—that requires vitamin K for its proper action has been obtained from livers of normal healthy cows (33) Studies of rat liver

the block of the vitamin K dependent carboxylation of Factor IX antigen

The present study shows that at birth both antigen and clotting activity of Factor IX are low and the ratio is 1 01 The post natal increase of the Factor IX during the first three days of life involves both antigen level and clotting activity and the ratio remains unchanged For the first 30 days of life the two activities of Factor IX remain constant at a level lower than normal adult values (adult norms for clotting activity 0 930±0 130 SI unit and for antigen level 0 930±0 190 SI unit —ratio 1 0) (30)

We have observed that the post natal increase of clotting activity of Factor IX is not statistically correlated with oral administration of vitamin K_1 at birth We have not obtained any evidence in the newborns of different populations of Factor IX molecules or of aberrant migration of the Factor IX protein in calcium containing buffer

In conclusion our findings show that newborn infants have a reduced synthesis of a normal Factor IX that vitamin K does not influence the amount of biologically active Factor IX after birth and that the ratio between clotting activity and antigen level is stable

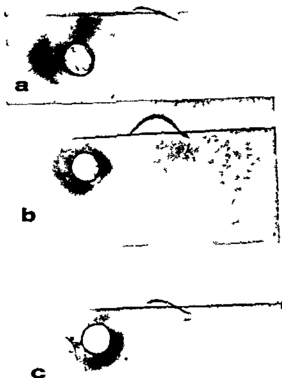


Fig 3 Crossed immunoelectrophoresis of Factor IX (in the first dimension run buffer contains 0 0025 M Ca^{2+}) (a) Newborn 5-day-old (after vitamin K) (b) Adult plasma pool (c) Newborn 5-day-old (without vitamin K)

slices showed that vitamin K is required for the formation of Factor VII from a polypeptide precursor (3 21)

The N terminal sequence of human Factor IX (9) and of bovine Factor IX (10) also contains gamma carboxyl glutamic acid residues Factor IX binds Ca^{2+} in a similar way as prothrombin (12) An abnormal circulating inactive Factor IX molecule has been shown in patients receiving vitamin K antagonists (30) All Warfarin treated patients had lower Factor IX clotting activity and the clotting/antigen ratio was 0 40 (30) In this condition the reduction of clotting activity was related to

ACKNOWLEDGEMENTS

We are grateful to Dr N Heimburger Behringwerke AG Aktiengesellschaft Marburg FRG for the generous supply of anti Factor IX serum

This study was supported by grants from C N R Rome (Contract no 75 00621 04) and from Department of Public Education Rome

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Submitted June 29 1978
Accepted July 2 1979

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Acta Pædiatr Scand 69 59–63, 1980

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ABSTRACT Ikonen, R S, Lindgren, J, Niemi E, Sorto A E, Seppala M and Ruoslahti E (Department of Paediatrics, Central Hospital of Tampere, Department of Bacterio- [illegible]

[illegible] Duarte California, USA) Alpha fetoprotein levels in neonatal hyperbilirubinaemia Acta Paediatr Scand 69 59, 1980.—Serum alpha fetoprotein (AFP) levels were studied in 15 neonatally hyperbilirubinaemic children and 15 controls matched for sex and gestational age All children were born between 38 and 40 weeks of gestation During the first seven weeks of postnatal life hyperbilirubinaemic children had serum AFP concentrations over twice as [illegible] and [illegible] mg/l ($p<0.001$) However, no correlation was found between serum bilirubin and AFP concentrations in hyperbilirubinaemic children

KEY WORDS Alpha fetoprotein, hyperbilirubinaemia, newborn

Alpha fetoprotein (AFP) is a major plasma protein in early fetal development (3) AFP synthesis takes place in the fetal liver, yolk sac and gastrointestinal tract (6) In fetal serum the AFP concentration is highest at the 13th week of gestation after which it decreases (5) At birth AFP is readily detectable in cord serum The time for the AFP level to decrease by 50% is 5 days during the first postnatal week and 3 days thereafter (5) The apparent half life of AFP in newborn serum is similar to that of maternal serum AFP during the postpartum period (18) The adult background level of 2–25 μg/l (14) is usually reached by the age of 2 years (8)

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Elevated AFP values in early infancy have been reported in many hepatic disorders (1, 7), especially in neonatal hepatitis (21, 2), and babies born to Rh immunized mothers have AFP levels which are higher than normal (17) In this report we present data showing that AFP levels are higher during the first seven weeks of life in children who had unconjugated neonatal hyperbilirubinaemia without hepatitis or hemolytic disease

MATERIALS AND METHODS

A prospective study of 15 [illegible] was determined from the

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MATERIALS AND METHODS

A prospective

Table 1 *Serum bilirubin, transaminase and alkaline phosphatase values in neonatally hyperb bilirubinaemic children*

No	Sex		5–7 days Bilirubin µmol/l Tot	Conj	ASAT (U/l)	AP (U/l)	20–25 days Bilirubin µmol/l Tot	Conj	ASAT (U/l)	(
1	Boy	(253)	230[a]	15 5	35	490	66	14 6	32	6
2	Girl		296	14 8	26	309	237	19 1	–	[illegible]
3	Girl	(270)	194[a]	5 0	76	252	19	13 1	47	[illegible]
4	Girl		274	14 4	26	322	121	16 3	56	[illegible]
5	Girl		283	12 5	45	438	181	20 0	40	5
6	Boy		336	14 8	–	–	224	12 9	43	[illegible]
7	Boy		287	17 3	69	–	124	14 3	35	6
8	Boy		259	14 0	32	284	–	–	37	5
9	Boy		305	19 3	25	523	264	27 0	20	6
10	Boy		296	21 6	41	432	181	26 8	32	6
11	Girl		269	14 0	48	441	250	6 0	32	6
12	Boy	(256)	240[a]	21 6	47	275	142	14 4	94	3
13	Boy		266	14 8	49	394	161	14 0	62	4
14	Girl		265	18 3	–	411	64	8 6	32	5
15	Boy	(253)	246[a]	29 3	17	540	230	15 8	28	8
Mean			270	16 5	41	393	162	15 9	42	5
S E M			8 8	1 4	4 8	26 8	20 4	1 6	5 0	

[a] The bilirubin values of these patients (estimated as described in 9) had fallen below 250 µmol/l at 5–7 days The bilirubin values (estimated by the micromethod) are shown in parentheses
[b] Thirteen out of the 15 children initially studied were available for observation at this age

last menstrual period and the difference in each pair did not exceed three days The gestational age (mean ± S E M) was 273±4 7 days in the hyperbilirubinaemic group and 273±4 4 days in the controls All children were healthy except for the hyperbilirubinaemia No infant with Rh or ABO hemolytic disease was included Exchange blood transfusion was not needed in any of cases Phototherapy was given to five of the hyperbilirubinaemic children

Serum aspartate aminotransferase (ASAT) alkaline phosphatase (AP) and bilirubin concentrations were estimated as described (20 9) and the serum AFP concentration was measured by double antibody radioimmunoassay (15) The sensitivity of the AFP assay was 0 25 µg/l and the inter assay coefficient of variation was 16% One µg unit of the International reference preparation for AFP (19) gave a value of 0 72 µg under the test conditions

Blood samples were taken at the age of 5–7 20–25 and 40–49 days There were no clinical signs of any abnormal [illegible] of AFP [illegible] and controls Correlation between individual bilirubin and AFP levels was studied by the Spearman correlation analysis

RESULTS

The serum bilirubin, ASAT and AP values in neonatally hyperbilirubinaemic children are given in Table 1 Most of the initially hyper bilirubinaemic children continued to show ar elevated serum bilirubin concentration at 40- 49 days The ASAT and AP activities were within normal limits (12), except in case no

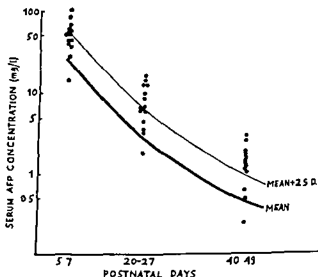


Fig 1 AFP levels in individual children with neonatal hyperbilirubinaemia (dots) The smooth curves represent the AFP levels in the controls

40–49 days[b]			
Bilirubin µmol/l		ASAT (U/l)	AP (U/l)
Tot	Conj		
16 4	5 8	53	1 072
–	–	–	–
29 6	15 2	48	621
47 8	14 1	62	–
114	11 8	58	664
88 9	9 0	–	637
14 2	4 8	44	781
–	–		–
77 0	25 0	22	443
66 7	10 9	24	676
157	18 0	52	865
96 0	12 9	107	506
21 6	9 0	52	727
6 8	2 5	25	625
55 2	11 9	27	605
60 8	11 6	48	685
12 2	1 6	6 8	47 4

12 where the ASAT activities were slightly elevated at the age of 20–25 and 40–49 days

A decreasing slope of serum AFP level with advancing age was found in all children (Fig 1) The AFP value exceeded two standard deviations of the mean AFP level of controls in 5 of 14 hyperbilirubinaemic infants at the age of 5–7 days, in 7 of 15 cases at 20–25 days, and in 10 of 13 cases at 40–49 days

The AFP values in hyperbilirubinaemic and control children are shown in Table 2 In two pairs (no 8 and 9) the AFP values were similar in hyperbilirubinaemic and control children whereas in all other cases the AFP levels were higher in hyperbilirubinaemic children At the age of 5–7 days the mean AFP levels for hyperbilirubinaemic infants and controls were 52 4 and 24 8 mg/l, respectively ($p<0\ 001$) At the age of 20–25 days the mean AFP values were 7 28 mg/l for hyperbilirubinaemic infants and 2 75 mg/l for controls ($p<0\ 001$), and at 40–49 days they were 1 39 and 0 46 mg/l, respectively ($p<0\ 001$) This difference was significant at all postnatal ages when the groups were matched for sex, and there was no significant difference in the AFP levels between boys and girls

There was no significant correlation between serum bilirubin and AFP levels in the hyperbilirubinaemic group

DISCUSSION

Our results show that neonates who experience hyperbilirubinaemia have higher AFP levels than controls matched for age, gestational age and sex, and that the difference in AFP levels between these two groups becomes more striking during the first seven weeks of postnatal life There were only two pairs in which no difference was noted in the AFP levels between hyperbilirubinaemic children and controls There also was some overlapping in the AFP values between hyperbilirubinaemic infants and controls as a group Hyperbilirubinaemia was unconjugated in all cases, and no evidence for neonatal hepatitis was found Elevated bilirubin values even at 40–49 days of life seem to be quite common in infants with neonatal hyperbilirubinaemia This can most probably be explained by the slow enzymatic maturation of the liver One contributing factor may also be breast feeding

It has previously been found that the AFP concentration in cord serum generally exceeds the normal median value in infants born with Rh hemolytic disease (17), and the present results extend this observation to neonatal hyperbilirubinaemia without associated hemolytic disease

The fetal serum AFP level decreases rapidly with advancing gestation, and the concentration continues to decrease after birth (5, 8) Thus, gestational age at birth is a critical determinant of the postnatal AFP concentration during the first weeks of life In this study, the controls were matched for gestational age

... level we ob-

Table 2 *Serum AFP values (mg/l) in neonatally hyperbilirubinaemic and control children*

		5–7 days		20–25 days		40–49 days	
No	Sex	Hyperbili rubinaemia	Con trols	Hyperbili rubinaemia	Con trols	Hyperbili rubinaemia	Con trols
1	Boy	27 20	14 50	8 64	3 82	1 34	1 21
2	Girl	38 75	9 50	6 19	1 15	–	0 23
3	Girl	100 00	28 38	12 50	1 88	3 00	0 43
4	Girl	–	20 65	6 20	4 69	1 50	0 55
5	Girl	83 50	33 25	15 50	3 07	1 54	–
6	Boy	53 75	30 00	9 50	1 63	2 69	0 32
7	Boy	14 87	2 28	3 25	0 58	0 50	0 27
8	Boy	53 13	53 75	4 34	5 57	–	0 59
9	Boy	56 25	58 34	6 13	6 13	0 65	0 56
10	Boy	60 00	25 66	6 82	3 53	1 40	0 47
11	Girl	43 13	13 25	5 88	3 63	1 18	0 42
12	Boy	38 75	11 13	1 82	1 02	1 65	0 28
13	Boy	44 69	31 88	14 38	2 20	1 12	–
14	Girl	52 50	22 50	3 44	1 27	0 26	0 23
15	Boy	67 50	13 50	12 75	1 19	1 21	0 47
Mean		52 4	24 8	7 28	2 75	1 39	0 46
S E M		5 8	4 3	1 10	0 45	0 21	0 07
		$t=3\ 812$ $p<0\ 001$		$t=4\ 27$ $p<0\ 001$		$t=4\ 09$ $p<0\ 001$	

served cannot be ascribed to a difference in *gestational ge*

AFP and albumin show homology in their amino acid sequences (16), and it has recently been shown that AFP, like albumin, binds bilirubin (13) It is not known how hyperbilirubinaemia is related to elevated AFP level The elevated levels of AFP and bilirubin may both reflect functional immaturity of the liver (4) but the possibility that the elevated AFP levels could somehow be related to a functional role of AFP in bilirubin metabolism can not be excluded at present

ACKNOWLEDGEMENTS

The authors wish to thank Ms Seija Nikunen and Ms Sirkka Soikkeli for technical assistance

Supported by grants from the Research Council for Medical Sciences The Academy of Finland (M S) the Paulo Foundation (R S I) and the National Foundat on March of Dimes (E R)

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Submitted April 6 1979
Accepted June 14 1979

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Table 2. *Serum AFP values (mg/l) in neonatally hyperbilirubinaemic and control children*

		5–7 days		20–25 days		40–49 days	
No	Sex	Hyperbili-rubinaemia	Con-trols	Hyperbili-rubinaemia	Con-trols	Hyperbili-rubinaemia	Con trols
1	Boy	27.20	14.50	8.64	3.82	1.34	1.21
2	Girl	38.75	9.50	6.19	1.15	–	0.23
3	Girl	100.00	28.38	12.50	1.88	3.00	0.43
4	Girl	–	20.65	6.20	4.69	1.50	0.55
5	Girl	83.50	33.25	15.50	3.07	1.54	–
6	Boy	53.75	30.00	9.50	1.63	2.69	0.32
7	Boy	14.87	2.28	3.25	0.58	0.50	0.27
8	Boy	53.13	53.75	4.34	5.57	–	0.59
9	Boy	56.25	58.34	6.13	6.13	0.65	0.56
10	Boy	60.00	25.66	6.82	3.53	1.40	0.47
11	Girl	43.13	13.25	5.88	3.63	1.18	0.42
12	Boy	38.75	11.13	1.82	1.02	1.65	0.28
13	Boy	44.69	31.88	14.38	2.20	1.12	–
14	Girl	52.50	22.50	3.44	1.27	0.26	0.23
15	Boy	67.50	13.50	12.75	1.19	1.21	0.47
Mean		52.4	24.8	7.28	2.75	1.39	0.46
S E M		5.8	4.3	1.10	0.45	0.21	0.07
		$t=3.812$ $p<0.001$		$t=4.27$ $p<0.001$		$t=4.09$ $p<0.001$	

served cannot be ascribed to a difference in gestational ge.

AFP and albumin show homology in their amino acid sequences (16), and it has recently been shown that AFP, like albumin, binds bilirubin (13). It is not known how hyperbilirubinaemia is related to elevated AFP level The elevated levels of AFP and bilirubin may both reflect functional immaturity of the liver (4) but the possibility that the elevated AFP levels could somehow be related to a functional role of AFP in bilirubin metabolism cannot be excluded at present

ACKNOWLEDGEMENTS

The authors wish to thank Ms Seija Nikunen and Ms Sirkka Soikkeli for technical assistance

Supported by grants from the Research Council for Medical Sciences, The Academy of Finland (M S) the Paulo Foundation (R S I), and the National Foundation March of Dimes (E R)

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Acta Pædiatr Scand 69 65–68, 1980

GUT TRANSIT TIME AND LACTOSE MALABSORPTION DURING PHOTOTHERAPY

I A Study Using Lactose-free Human Mature Milk

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From the [1]Department of Neonatology Rigshospitalet and the [2]Department of Dairy Chemistry and Dairy Bacteriology The Royal Veterinary and Agricultural University Copenhagen Denmark

ABSTRACT Ebbesen, F , Edelsten, D and Hertel, J (Department of Neonatology, Rigshospitalet and Department of Dairy Chemistry and Dairy Bacteriology, Royal Veterinary and Agricultural University, Copenhagen, Denmark) Gut transit time and lactose malabsorption [illegible]

milk) was added 30 infants received ordinary phototherapy and 30 intensive phototherapy (blue double light) 15 in each group had "sucrose milk" and 15 "lactose milk" There was no significant difference between the increase in blood glucose (ΔBS) by lactose tolerance tests performed before phototherapy (LTT) and by those performed [illegible]
neither in infa [illegible]
normal ΔBS-L [illegible]
difference in g [illegible]
"lactose milk" [illegible]
transit time was significantly shorter in infants treated with intensive phototherapy than in infants treated with ordinary phototherapy without there being any significant difference in [illegible]

KEY WORDS *Phototherapy, lactose malabsorption, gut transit time, newborn infants, hyperbilirubinemia*

Frequent loose green stools are often observed during ordinary phototherapy of newborn infants with hyperbilirubinemia and in the vast majority of infants during intensive phototherapy (blue double light) (5 6), beginning a few hours after the start of the treatment The gut transit time (GTT) measured during ordinary phototherapy is reduced (12, 14) Bakken (2) examined six infants who developed diarrhoea during ordinary phototherapy and found that all of them had a temporary lactase deficiency Their lactose tolerance tests (LTT) were flat and no lactase activity was found in intestinal biopsies The author concluded that the reduced GTT during phototherapy is due to a temporary lactase deficiency This deficiency was supposed to be caused by unconjugated bilirubin, since this can *in vitro* inhibit intestinal lactase (2) and since it is found in the gut in increased amounts during phototherapy (4, 8, 11)

The purpose of the present study was to investigate whether lactose malabsorption is a common phenomenon during phototherapy, and thus accounts for the reduced GTT in general This is of great clinical importance in deciding whether or not infants developing diarrhoea during phototherapy should be fed with lactose free milk, as advocated by Bakken (2)

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ABSTRACT [illegible] plicated hyperbilirubinemia were studied They were fed human mature milk from which lactose had been eliminated, whereafter either sucrose ("sucrose milk") or lactose ("lactose milk") was added 30 infants received ordinary phototherapy and 30 intensive phototherapy (blue double light) 15 in each group had "sucrose milk" and 15 "lactose milk". There was no significant difference between the increase in blood glucose (ΔBS) by lactose tolerance tests performed before phototherapy (LTT_I) and by those performed during phototherapy (LTT_{II}), neither in infants treated with ordinary nor with intensive [illegible] normal ΔBS-L [illegible] difference in g [illegible] "lactose milk" [illegible] transit time wa [illegible] infants treated [illegible]

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ABSTRACT Ebbesen, F , Edelsten, D and Hertel, J (Department of Neonatology, Rigshospitalet and Department of Dairy Chemistry and Dairy Bacteriology, Royal Veterinary and Agricultural University, Copenhagen, Denmark) Gut transit time and lactose malabsorption during phototherapy I A study using lactose free human mature milk Acta Paediatr Scand, 69 65, 1980.—Sixty newborn infants with normal birth weight suffering from uncomplicated hyperbilirubinemia were studied They were fed human mature milk from which lactose had been eliminated, whereafter either sucrose ("sucrose milk") or lactose ("lactose

performed before phototherapy (LTT$_I$) and by those performed during phototherapy (LTT$_{II}$) neither in infants treated with ordinary nor with intensive phototherapy All infants had normal ΔBS-LTT$_I$ except one receiving ordinary phototherapy There was no significant d fferen

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Table 2 *Rise in blood glucose concentrations by lactose tolerance tests and gut transit times*

	Ordinary phototherapy		Intensive phototherapy	
	Sucrose milk Grup I (n=15) *Median (range)*	Lactose milk Group II (n=15) *Median (range)*	Sucrose milk Group III (n=15) *Median (range)*	Lactose milk Group IV (n=15) *Median (range)*
ΔBS-LTT$_I$ (mmol/l)[a]	2 6 (1 1–6 1)	3 2 (1 8–5 8)	–	–
ΔBS LTT$_{II}$ (mmol/l)[b]	2 8 (1 7–5 0)	3 2 (1 0–5 6)	2 8 (1 4–3 5)	2 9 (1 8–6 4)
GTT (hours)[c]	10 5 (4 0–29 2)	8 0 (4 0–23 8)	6 8 (2 0–15 0)	6 5 (3 3–13 4)

[a] Rise in blood glucose concentrations by lactose tolerance tests performed before phototherapy
[b] Rise in blood glucose concentrations by lactose tolerance tests performed during phototherapy
[c] Gut transit times

of the infants it was investigated whether a correlation existed between ΔBS-LTT$_I$ and the hour of life at which the LTT$_I$ was performed Such a correlation was not found (Spearman s rank correlation coefficient −0 032 $p>0$ 4)

RESULTS

The results are presented in Table 2 There was no significant difference between ΔBS LTT$_I$ and ΔBS LTT$_{II}$ in group I and in group II (infants receiving ordinary phototherapy) Nor was there any significant difference when ΔBS LTT$_I$ in groups I + II was compared with ΔBS LTT$_{II}$ in group III and in group IV (infants receiving intensive phototherapy) Nor was there any significant difference between ΔBS LTT$_I$ and ΔBS LTT$_{II}$ when infants treated with ordinary phototherapy were considered jointly or when the infants treated with intensive phototheray were considered jointly ($p>0$ 3 in all tests)

Two infants treated with ordinary phototherapy had a flat LTT In one case the LTT was flat before phototherapy (ΔBS LTT$_I$=1 1 mmol/l) but normal during the treatment (ΔBS LTT$_{II}$=3 9 mmol/l) In the other case the LTT was normal before the phototherapy (ΔBS LTT$_I$=2 2 mmol/l) but flat during the treatment (ΔBS LTT$_{II}$=1 0 mmol/l) This infant had lactose milk , GTT was 4 3 hours, and the infant had 6 normal to loose stools for the last 24 hours before the first carmin coloured stool was recovered

There was no significant difference in GTT between the infants having "sucrose milk" and the infants having "lactose milk" , neither in those treated with ordinary phototherapy nor in those treated with intensive phototherapy ($p>0$ 6)

GTT was significantly shorter in infants threated with intensive phototherapy than in infants treated with ordinary phototherapy ($p<0$ 05) without there being any difference in ΔBS LTT$_{II}$ ($p>0$ 6) (GTT was median 6 7, range 2 0–15 0 hours (n=30) and median 8 7, range 4 0–29 2 hours (n=30), respectively)

DISCUSSION

GTT during phototherapy is dependent on light intensity, since GTT was significantly shorter in infants treated with intensive phototherapy than in those treated with ordinary phototherapy This is in agreement with the greater reduction rate of the plasma bilirubin concentration by intensive phototherapy than by ordinary phototherapy, corresponding to a higher excretion with the bile of unconjugated bilirubin and bilirubin decomposition derivatives, which presumably are the substances causing the reduced GTT (12) according to Bakken (2) through a lactase defiency

A direct estimation of the intestinal lactase activity was not performed, but the ΔBS LTT correlates well with the lactase activity in newborn infants (2) We found no evidence for

Table 1. *Data of the patients*

	Ordinary phototherapy		Intensive phototherapy	
	"Sucrose milk" Group I ($n=15$) Median (range)	"Lactose milk" Group II ($n=15$) Median (range)	"Sucrose milk" Group III ($n=15$) Median (range)	"Lactose milk Group IV ($n=15$) Median (range)
Birth weight (g)	3 300 (2 510–4 000)	3 300 (2 900–4 400)	3 500 (2 660–4 330)	3 430 (2 750–4 000)
Gestational age (weeks)	39 (36–42)	40 (37–42)	39 (35–42)	39 (36–42)
Age at start of phototherapy (hours)	88 (72–132)	89 (54–134)	82 (58–124)	87 (60–111)
Duration of phototherapy (hours)	61 (46–71)	61 (42–89)	32 (20–38)	25 (22–39)
Initial plasma bilirubin concentration (μmol/l)	265 (217–332)	276 (204–326)	287 (259–302)	279 (251–303)
Decrease in plasma bilirubin concentration during phototherapy (μmol/l)	86 (43–114)	101 (34–130)	122 (63–181)	118 (73–159)
Decrease in plasma bilirubin concentration per hour during phototherapy (μmol/l/h)	1 5 (0 9–2 4)	1 5 (0 5–2.7)	4 0 (2 4–6 0)	4 1 (2 5–5 2)

MATERIALS AND METHODS

Sixty light treated newborn infants with normal birth weight (>2 500 g) suffering from uncomplicated hyperbilirubinemia, were consecutively included in the study Their direct Coombs' tests were negative The first 30 infants received ordinary phototherapy (groups I and II) and the next 30 infants intensive phototherapy (blue double light) (5, 6) (groups III and IV) By ordinary phototherapy the infants were exposed to light from above with a mixture of "daylight" and blue light The light intensity was 6 5 W/m² (B) 10 cm above the mattress level measured with a photometer (9) By intensive phototherapy the infants were exposed to blue light from both above and below The light intensity from above was 9 5 W/m² (B) 10 cm above the mattress and from below 10 5 W/m² (B) at mattress level Each infant was treated continuously throughout the investigation Informed consents were obtained from the mothers

The infants were fed with human mature milk from which the lactose was eliminated by fermentation with Saccharomyces fragilis[1] (7), whereafter either sucrose ("sucrose milk") or lactose ("lactose milk") was added to 7 0% (w/v) Infants with even record numbers had "sucrose milk" (groups I and III) and infants with uneven numbers had "lactose milk" (*groups II and IV*) *The* amount of milk given depended on age and weight according to a standard regime

GTT was measured during phototherapy in each infant from administration of 1 0 ml 5% carmin solution to recovery of the first red stool The carmin solution was given 36 hours after start of ordinary phototherapy and 12 hours after start of intensive phototherapy in order to ensure approximately *the same decrease* of the plasma bilirubin concentration at the time of carmin administration The carmin was given by gastric tube just before the meal

A lactose tolerance test was made before phototherapy (LTT_I) in the first 30 infants (groups I and II) and during phototherapy (LTT_{II}) in all infants after the first carmin coloured stool had been recovered A LTT was made after four hours' fasting by giving 2 0 g lactose/kg body weight in a 10% solution by means of a gastric tube and measuring the blood glucose concentration at 0 30 45, 60 [illegible] An increase [illegible]

not performed since we found it unethical to perform intestinal biopsies on newborn infants with uncomplicated hyperbilirubinemia

The blood glucose concentration was measured by a glucose dehydrogenase method (3) and the plasma bilirubin concentration by a direct spectrometric method (13) on capillary blood

Statistical analyses were performed using Mann Whitney's test for two independent samples and Wilcoxon's test for paired observations

The data for the four groups of infants are summarized in Table 1 There was no significant difference in regard to birth weight, gestational age age at start of phototherapy, and initial plasma bilirubin concentration The reduction in plasma bilirubin concentration per hour was significantly greater in infants treated with intensive phototherapy than in those treated with ordinary phototherapy ($p<0\ 001$)

To ensure that ΔBS LTT was independent of the age

[1] The yeast Saccharomyces fragilis fermented the lactose to carbon dioxide and ethanol, which were removed by vacuum distillation The yeast cells were removed by centrifugation

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II A Study Using Raw Milk from the Mothers of the Infants

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ABSTRACT Ebbesen F, Ed ...

... being any significant difference in the increase in blood glucose by lactose tolerance tests It is concluded that lactose malabsorption is not the usual cause of the reduced gut transit time during phototherapy even if the infants receive their mothers' milk.

KEY WORDS Phototherapy, lactose malabsorption, gut transit time, newborn infants, hyperbilirubinemia

Bakken (1) examined six infants who developed diarrhoea during ordinary phototherapy and found that all of them had a temporary lactase deficiency These infants received their mothers' milk The author concluded, that the reduced gut transit time (GTT) during phototherapy is caused by this deficiency In contrast, we found that usually the reduced GTT during phototherapy is *not* due to a lactose malabsorption, neither in infants treated with ordinary phototherapy nor in infants treated with intensive phototherapy (2) In our study, the infants received pasteurized human mature milk from which the lactose had been removed by fermentation, whereafter sucrose or lactose was added to 7 0% (w/v) This milk is biochemically somewhat different from their mothers' milk (transitional milk) in regard to protein, fat, electrolytes etc. In order to exclude that the different results of Bakken and our investigations were due to the fact that the infants received different milk, i e that the inhibition of the lactase activity only occurred when the infants had their mothers' milk, we continued our investigations using the raw milk from the mothers of the infants

MATERIALS AND METHODS

Thirty light treated newborn infants, fulfilling the same criteria as the infants in our former investigation (2) were consecutively included in the study 15 infants received ordinary phototherapy and 15 infants intensive phototherapy (blue double light) (2) All infants received their mothers milk or fresh milk from mothers of other new ...

... (2) An increase in blood glucose ...

... concentrations were determined as de-

lactose malabsorption being the usual cause of the reduced GTT during phototherapy, neither in infants treated with ordinary phototherapy nor in infants treated with intensive phototherapy (*a*) ΔBS-LTT was the same before and during phototherapy, (*b*) all infants, with one exception, had normal LTT during phototherapy, (*c*) GTT was independent of whether the milk contained sucrose or lactose, and (*d*) GTT was significantly shorter in the infants treated with intensive phototherapy than in the infants treated with ordinary photo therapy without there being any difference in ΔBS-LTT during the phototherapy

One infant showed a flat LTT during phototherapy This infant may have developed a lactose malabsorption, since ΔBS-LTT was normal before the phototherapy and GTT was short during the treatment However, the LTT might as well have been false positive, since in another infant we found a false positive LTT before the phototherapy, and it is known that false positive LTT occurs when the lactose is installed in the stomach (10), probably due to slow gastric emptying rate In one infant a lactose malabsorption might thus have been contributory to the short GTT That the LTT was false positive in the other infant was evident from the high ΔBS-LTT 48 hours later during phototherapy Such a rapid develop ment of the lactase activity will hardly occur, and in infants born at term the lactase activi ty has also been found to be fully developed at birth (1)

Thus, it can be concluded that lactose malabsorption is not the usual cause of the reduced GTT during phototherapy and that such a malabsorption must be a rare complication in phototherapy

ACKNOWLEDGEMENTS

The authors wish to thank the staff of the Department of Neonatology and the Department of Clinical Chemistry ML and the dieticians for helpful assistance

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Submitted April 23 1979
Accepted July 10 1979

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Acta Pædiatr Scand 69 69–71, 1980

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KEY WORDS Phototherapy, lactose malabsorption, gut transit time, newborn infants, hyperbilirubinemia

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GTT wa
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(2) An increase in blood glucose concentration (ΔBS) at LTT less than 1 2 mmol/l (flat curve) was considered abnormal A direct estimation of the intestinal lactase activity was not performed due to ethical reasons Blood glucose and plasma bilirubin concentrations were determined as de

Table 1 *Data of the patients*

	Ordinary phototherapy (n=15) Median (range)	Intensive phototherapy (n=15) Median (range)
Birth weight (g)	3 370 (2 850–4 550)	3 300 (2 910–4 050)
Gestational age (weeks)	39 (37–42)	40 (34–43)
[illegible]	92 (55–172)	89 (52–108)
[illegible]	62 (45–70)	24 (16–50)
[illegible]	269 (237–303)	280 (226–323)
[illegible] during phototherapy (μmol/l)	82 (3–121)	96 (52–157)
Decrease in plasma bilirubin concentration per hour during phototherapy (μmol/l/h)	1 2 (0 1–2 6)	4 3 (2 0–5 8)

scribed (2) Informed consents were obtained from the mothers Statistical analyses were performed using the Mann Whitney's test Data for the two groups of infants are summarized in Table 1 There was no significant difference in regard to birth weight gestational age age at start of phototherapy, and initial plasma bilirubin concentration The reduction in plasma bilirubin concentration per hour was significantly greater in infants treated with intensive phototherapy than in those treated with ordinary phototherapy (p<0 001)

RESULTS

All infants had a normal LTT during the phototherapy, with the exception of one infant. This infant received ordinary phototherapy, the ΔBS-LTT was 1 0 mmol/l, the GTT was 9 4 hours, and the infant had five normal stools for the last 24 hours, before the first carmin coloured stool was recovered

GTT was significantly shorter in the infants treated with intensive phototherapy than in those treated with ordinary phototherapy (p<0 01) without there being any significant difference in ΔBS-LTT (Table 2)

Table 2 *Rise in blood glucose concentrations by lactose tolerance tests and gut transit times*

	Rise in blood glucose by lactose tolerance tests (mmol/l) Median (range)	Gut transit times (hours) Median (range)
Ordinary phototherapy (n=15)	2 3 (1 0–3 3)	9 0 (3 3–17 4)
Intensive phototherapy (n=15)	2 8 (1 7–5 0)	3 3 (2 5–17 8)

DISCUSSION

The investigation confirmed that during phototherapy the GTT is dependent on the light intensity (2), since GTT was significantly shorter in the infants treated with intensive phototherapy than in those treated with ordinary phototherapy

A direct estimation of the intestinal lactase activity was not performed, but ΔBS-LTT correlates well with the lactase activity in newborn infants (1) Even if the infants received their mothers' milk there was no evidence for lactose malabsorption being the usual cause of the reduced GTT during phototherapy, neither during ordinary nor during intensive phototherapy None of the infants developed a lactose malabsorption during the phototherapy—the only flat LTT was a false positive, since GTT was relatively long—and the very short GTT in the infants treated with intensive phototherapy compared with that of the infants treated with ordinary phototherapy was not due to a lactose malabsorption, as there was no difference in ΔBS-LTT between the two groups and all infants treated with intensive phototherapy had normal LTT

Thus, our two investigations showed that

during phototherapy the reduced GTT is not usually caused by lactose malabsorption and that such a malabsorption must be a rare complication in phototherapy even if the infants are exposed intensively to light

ACKNOWLEDGEMENTS

The authors wish to thank the staff of the Department of Neonatology and the Department of Clinical Chemistry ML for helpful assistance

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ACTIVITY OF THE INTERNAL ANAL SPHINCTER DURING THE FIRST DAYS OF LIFE

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From the Department of Paediatric Surgery St Goran s Hospital Stockholm Sweden

ABSTRACT [illegible]

Internal spincter relaxation was recorded in all infants but one on the first day, in all on the second and finally in all but one on the third and fourth days The results indicate that ano-rectal manometry may be used in the diagnosis of Hirschsprung's disease also in the newborn period However, repeated examinations may be necessary and the diagnostic reliability may be somewhat lower than in older children

KEY WORDS Ano-rectal manometry, Hirschsprung s disease, neonates

Normally, there is a relaxation of the internal sphincter upon rectal distension (4, 12) This reflex is mediated via ganglion cells in the rectal wall During fetal life the development and maturation of the ganglion cells proceed in a cranio-caudal direction At birth ganglion cells are present in the rectum, but they may show signs of immaturity (18) Thus, the activity of the internal sphincter may be expected *to be different in the neonatal period* Some authors have found atypical activity and absence of relaxation reflex during the first days of life in premature as well as in full term infants (14 15) while others have recorded a relaxation reflex right from the first day of life (2 3 22)

In Hirschsprung s disease, the ganglion cells are absent and the internal sphincter fails to relax upon rectal distension (16, 17) Manometric recordings of the internal anal spincter activity are frequently used in the diagnosis with satisfactory reliability (1, 9, 13, 20, 21) Knowledge of the normal physiology is of course essential and the aim of the present investigation was to study the internal sphincter activity during the first days of life

MATERIAL

The study was performed on 17 consecutively born healthy fullterm bab[illegible]

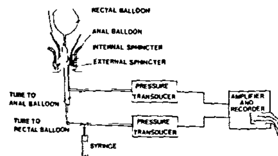


Fig 1 Diagrammatic representation of the method with an antero posterior view of the rectal and anal balloons in [illegible]

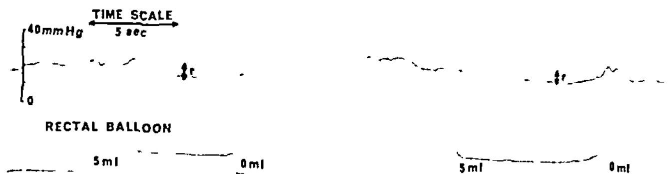


Fig 2 Recording of anal pressure during rectal distension in a 4 day old girl In response to each rectal inflation there is a decrease of anal pressure (indicated with r) due to a relaxation of the internal anal spincter

Swedish standards, except in two children who were slightly underweight and in one child who was slightly overweight Of the deliveries, 15 were normal (5 in epidural anaesthesia, 10 performed with pudendal block), one involved vacuum extraction with epidural anaesthesia because of exhaustion and one cesarean section indicated by narrowness All the infants had Apgar scores between 8 and 10 at one minute and 10 at five minutes

The meconium was passed before or during the first examination by all the babies except one, who did so at the second ano-rectal manometric examination

Having been informed in advance of the nature and purpose of the study, the parents gave their personal consent to it

METHODS

Equipment (Fig 1) The cuff of a Portex endotracheal tube No 5 0 was used to record the anal pressure The cuff was reduced to a length of about 1 cm by tying a silk ligature around the distal end The cuff was filled with water and connected to the recording equipment via a thin polyethylene tube The amount of water in the system was adjusted so that the pressure inside the cuff was equal to atmospheric pressure, but still kept the cuff expanded Rectal distension was achieved by a latex balloon with a length of 1 5–2 0 cm when not inflated The balloon was connected to a polyethylene tube length 50 cm inner diameter 2 mm and external diameter 2 4 mm This tube passed through the Portex tube and was connected to the recording equipment via a three-way stop cock The Portex tube and the rectal balloon were held in position manually by an assistant

The pressure in the rectal balloon and that in the cuff were recorded using two pressure regulated transducers (Siemens-Elema EMT 34) The signals were amplified (Siemens Elema EMT 31) and visualized by an ink jet recorder (Siemens Elema Mingograf 34)

The relaxations of the internal anal sphincter were defined quantitatively as the difference between the resting anal pressure and the smallest anal pressure recorded during the rectal expansion (Fig 2)

Procedure All the children were examined within 24 hours after birth and then daily during the following three days

During the examination the child was lying on its back The hips and knees were kept in a flexed position by the assistant using the right hand With the other hand the assistant held the tubes in position so that the cuff of the Portex endotracheal tube was placed at the level of the internal anal sphincter Inflations of the rectal balloon were performed, using two pressure levels, obtained by inflating 5 and 10 ml of air, respectively During each examination several inflations were made at both pressure levels

If irritated, the child was left for a while for nursing feeding or sleeping, so that the examination could always take place with the child in a seemingly relaxed state

Statistics Standard statistical methods were used When comparing means between several groups, one way analysis of variance was used (5) Data in the text and tables are given as mean ± S E (standard error of the mean) unless otherwise stated

RESULTS

Resting anal pressure (Table 1) There was no significant difference between the resting anal pressure levels obtained on the different days

Table 1 *Resting anal pressure during the first four days of life*

Day	Pressure	
1	3 5±0 16 kPa	26±1 2 mmHg
2	3 5±0 39 kPa	26±2 9 mmHg
3	3 2±0 32 kPa	24±2 4 mmHg
4	3 2±0 25 kPa	24±1 9 mmHg
Mean	3 3 kPa	25 mmHg

Table 2 *Relaxation of the internal spincter after rectal distension of 5 and 10 ml during the first four days of life*

Day	Relaxation			
	5 ml rectal distension		10 ml rectal distension	
1	0 89±0 11 kPa	6 7±0 83 mmHg	1 3±0 15 kPa	9 5±1 1 mmHg
2	1 0 ±0 13 kPa	7 7+0 98 mmHg	1 3±0 25 kPa	9 8+1 9 mmHg
3	0 87±0 17 kPa	6 5±1 3 mmHg	1 1±0 23 kPa	8 5±1 7 mmHg
4	1 1 +0 19 kPa	8 2±1 4 mmHg	1 6±0 29 kPa	12 ±2 2 mmHg
Mean	0 97 kPa	7 3 mmHg	1 3 kPa	9 6 mmHg

The overall mean anal pressure was 3 3 kPa (25 mmHg) The lowest individual pressure, 0 8 kPa (6 mmHg) was found on the third day in a child who frequently passed loose stools at the time of the examination

Internal sphincter relaxation (Fig 2, Table 2) At the first examination, performed within 24 hours after delivery, all infants but one showed a relaxation of the internal anal spinc ter upon a rectal distension of either 5 or 10 ml The infant in which relaxation was not re-corded had a resting anal pressure of 4 kPa (30 mmHg) On the second day, internal sphincter relaxation was recorded in all babies On the third and fourth days one child —not the same as on the first day—failed to relax the internal sphincter upon rectal dis tension (resting anal pressure 2 kPa – 15 mmHg on both occasions) He was re examined at one month, when normal relaxations were found On the fourth day another child showed proper relaxations in three consecutive infla tions He then had a large defecation and in flations after that just gave unreliable relaxa tions of about 0 2 kPa (1 5 mmHg) compared to about 1 kPa (7 5 mmHg) before the defeca tion Resting anal pressure in this child de creased from 2 7 kPa (20 mmHg) to 1 6 kPa (12 mmHg) after the defecation suggesting a decrease of internal sphincter tone

The relaxations averaged 0 97 kPa (7 3 mmHg) with 5 ml in the rectal balloon and 1 3 kPa (9 6 mmHg) with 10 ml in the rectal bal loon No significant difference was observed between the different days

Maximum relaxation was reached within an average of 3 4 sec (range 1–7 sec) after the rectal balloon had been inflated No difference between the four days was observed in this respect either

DISCUSSION

The pressure recorded within the anal canal at rest is predominantly generated by the inter-nal anal sphincter (6, 10) and it therefore large-ly reflects the activity of this sphincter In the present investigation, resting anal pressure did not differ between the first four days of life and averaged 3 3 kPa (25 mmHg) Conse quently, the tone of the internal sphincter does not seem to change in general during the first days of life In previous investigations (Table 3) resting anal pressure during the first year of life was found to average 6 3 kPa (47 mmHg) compared to 9 5 kPa (71 mmHg) and 10 1 kPa (76 mmHg) in older children (11) The latter does not differ from adult values (10) Thus, the results suggest that the internal sphincter tone is rather low after birth and then gradu ally increases reaching adult values after a

Table 3 *Resting anal pressure at different ages*

Neonatal	3 3 kPa	25 mmHg (present study)
0– 1 years	6 3 kPa	47 mmHg (9)
5– 7 years	9 5 kPa	71 mmHg (11)
10–12 years	10 kPa	76 mmHg (11)
Adult	8 5 kPa	64 mmHg (10)

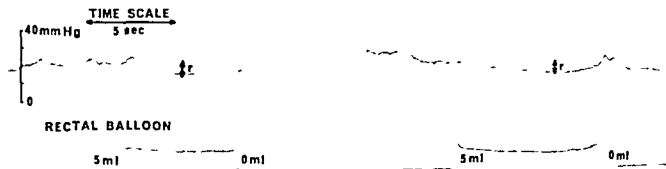


Fig 2 Recording of anal pressure during rectal distension in a 4 day old girl In response to each rectal inflation there is a decrease of anal pressure (indicated with r) due to a relaxation of the internal anal spincter

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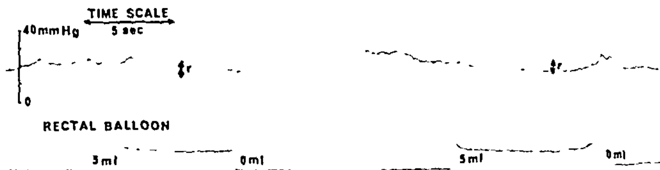


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Submitted May 12 1979
Accepted June 15 1979

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few years, i e when anal continence is fully developed

Despite the great individual variety in the resting anal pressure, it seems to depend on a few criteria Boston et al (2) showed a significant decrease of resting anal pressure when meconium had passed The only child in this investigation who did not pass meconium until the second day showed a similar decrease of anal pressure to half compared with the first day A prolonged relaxation of the internal sphincter was seen in one child who had a large defecation during the examination After this, anal pressure was abnormally low (1 6 kPa, 12 mmHg) and only very small relaxations could be recorded We also found a very low anal pressure in a child with abnormally watery stools at the time of the investigation This suggests that the state of bowel function may also influence the tone of the internal anal sphincter

Relaxations of the internal anal sphincter in response to rectal distension were recorded in all babies However, in one infant, no relaxation could be elicited on the first day and in another infant not on the third and fourth days Quantitatively, the relaxations did not differ between the first four days of life, but in general they were less pronounced than has been reported for older children (11), probably due to less sphincter tone, as discussed above Consequently, the present investigation suggests that the immaturity of the ganglion cells in the newborn (18) does not preclude the recto sphincteric reflex, but may contribute to the weakness of the internal sphincter tone and reflex

The present results indicate that ano rectal manometry may be used in the diagnosis of Hirschsprung's disease even in the newborn as the internal sphincter relaxation could be recorded in all children However, the fact that the recto sphincteric reflex could not be elicited at all four examinations in two infants indicates that repeated examinations may be necessary and that the diagnostic reliability may be less than in older children

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Acta Pædiatr Scand 69 79–82, 1980

DIAGNOSTIC VALUE OF SUCROSE TOLERANCE TEST IN CHILDREN EVALUATED BY BREATH HYDROGEN MEASUREMENT

A C DOUWES,[1] J FERNANDES and A A JONGBLOED

From the Department of Paediatrics Sophia Children's Hospital and Neonatal Unit Academic Hospital of the Erasmus University, Rotterdam the Netherlands

ABSTRACT. Douwes, A. C , Fernandes, J , Jongbloed, A. A (Sophia Children's Hospital and Neonatal Unit, Academic Hospital of the Erasmus University, Rotterdam, The Netherlands) [illegible] **compared to lactase In contrast to the lactose tolerance test, sucrose tolerance test should not be used as a screening procedure for secondary disaccharidase deficiency in children**

KEY WORDS Sucrose tolerance test, hydrogen breath test

The presence of nonabsorbed sugars in the gut lumen may produce diarrhea, meteorismus and abdominal pains (7, 13) and since dietary restriction of these sugars is beneficial, procedures have been developed to examine the tolerance to selected sugars The oral lactose tolerance test (LTT) and the oral sucrose tolerance test (STT) are the most widely used procedures and paediatricians rely on flat blood glucose curves to assess the presence of malabsorption and start elimination diets The challenge dose and the arbitrarily chosen lowest normal value for the rise in blood glucose is the same in LTT and STT (6)

Theoretically, this may affect the diagnostic value of the STT since the activity of sucrase in the brush border is about twice as high as lactase In order to evaluate this, we studied children and infants with intermittent diarrhea and/or abdominal pains by serial estimation of expired hydrogen (H_2) and rise in blood glucose during the STT and LTT.

PATIENTS AND METHODS

An STT was done in 103 patients, ages ranging from 3 months to 15 years Most of them had episodic diarrhea and/or abdominal pains of undetermined cause, some suffered from cystic fibrosis, inflammatory bowel disease, gluten sensitive enteropathy or genetic lactase deficiency The results of the LTT in these patients have been reported previously (4)

Sucrose (2 g/kg maximum 50 g 20% solution) was given orally at 9 a m after an overnight fast Blood glucose was est[illegible] [illegible] with sucrose intolerance were biopsied for microscopic examination of the duodenal mucosa and

[1] Present adress Children's Department Academic Hospital of the Free University, Amsterdam

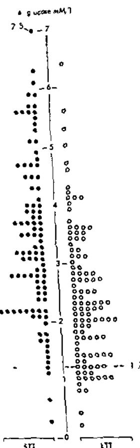


Fig 4 Δ Glucose in tolerant subjects (no abnormal H_2 increase) during an STT (n=100) and during an LTT (n=109)

viously (4) The mean value for Δ glucose in the sucrose tolerant group is significantly higher ($p<0.0001$) than in the lactose tolerant group (Table 1)

The occurrence of false flat glucose curves in the STT and LTT groups is 3% and 12 8% respectively Since the 95% confidence limits of these percentages do not overlap, the lower incidence of false flat blood glucose curves in the STT group is significant ($p<0.05$)

Sucrase activity in the duodenal mucosa of 11 sucrose tolerant children ranged from 3 9–8 8 μmoles sucrose split per minute per g mucosa (normal ≥1 9) Another sucrose tolerant child with untreated gluten sensitive enteropathy had 0 9 sucrase activity

Table 1 *Δ Glucose in mmol/l during lactose and sucrose tolerance test in subjects tolerant for these sugars*

Δ Glucose	Sucrose tolerant (n 100)	Lactose tolerant (n=109)
Mean and S D	3 42±1 43	2 43±1 17 (s)*
95% confidence limits	3 14–3 71	2 21–2 65
Range	0 3 –7 5	0 2 –6 4
% false	3	12 8 (s)**
95% confidence limits	1 5 –8 0	8 8 –18 7

* $p<0.0001$, ** $p<0.05$

DISCUSSION

There are a few reports on sucrose malabsorption (SM) and these are limited to sucrase-isomaltase deficiency (1, 10) Secondary SM is also rare and only occurs with severe enteropathy, short bowel syndrome and bacterial contamination of the small intestine (unpublished observations) and has not been reported in post gastroenteritis enteropathy In fact, children with acute gastroenteritis can be treated by oral administration of sucrose-saline solutions (8, 11) In the present study only 3 out of 103 children (1%) appeared to have SM in contrast to 54 of 163 (33%) having lactose malabsorption (4) We did not find secondary SM Even the patient with sucrase deficiency due to gluten sensitive enteropathy was found to be sucrose tolerant A similar finding has been reported earlier (6)

The rare occurrence of secondary SM may be explained by the finding that the brush border sucrase activity is about twice as high as lactase (9) Furthermore, this high sucrase activity probably accounts for the low number of false flat glucose curves during an STT Surprisingly, this has not been taken in consideration since challenge dose and definition of normal Δ-glucose are the same in STT and LTT (6) The present data show that during an STT mean Δ glucose is higher and the chance of finding a false flat glucose curve lower than during an LTT This contrasts with an earlier



Fig 1 Sampling device for mixed expired air for all ages The anaesthetic mask with lateral low resistance inlet valve is used for infants Older children may expire directly into the collection pipe

quantitative assay of the disaccharidases (2 12) Disaccharidase activity of 12 sucrose tolerant children was estimated because of an abnormal LTT A sucrase activity of ≥1 9 µmoles sucrose split per minute per g mucosa was considered normal (12)

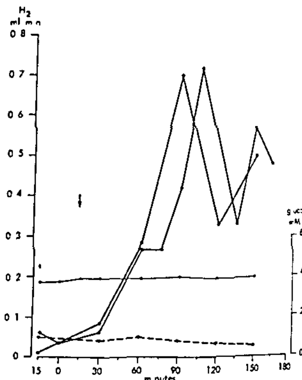


Fig 3 H_2 excretion (●—●) and blood glucose curve (●—●) during two subsequent STT's and H_2 excretion (● ●) after challenge with the monosaccharides of sucrose in 6-year old boy with sucrase isomaltase deficiency (rebreathing method)

RESULTS

Of the 103 children studied, 100 had normal H_2 excretion during the STT, indicating that no sucrose malabsorption occurred Two of 44 children asked for clinical symptoms during the STT however had abdominal pains and 1 was flatulent However, since stools passed during or shortly after the test revealed normal values for pH reducing substances and lactate these children were considered sucrose tolerant

The 3 children with abnormal excretion of H_2 had diarrhea and abdominal cramps but Δ glucose was normal in 2 of them Fig 2 illustrates our findings in one of these The normal H_2 excretion in one of these sucrose intolerant patients after administration of equivalent amounts of glucose and fructose demonstrated that the basic defect was in the hydrolysis of sucrose (Fig 3) This was confirmed by assay of the small bowel disaccharidases all 3 patients had congenital sucrase isomaltase deficiency

Fig 4 shows the Δ glucose of the 100 sucrose tolerant children and the Δ glucose in 109 lactose tolerant children reported pre

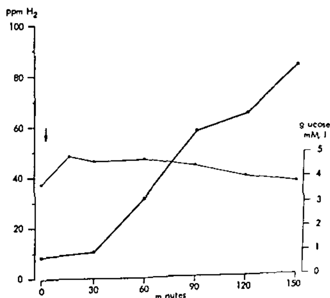


Fig 2 H_2excretion (●—●) and blood glucose curve (●—●) during an STT in a 5 year old boy with sucrase isomaltase deficiency (mixed expiratory air method)

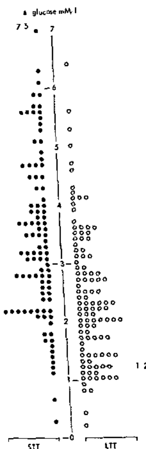


Fig 4 Δ Glucose in tolerant subjects (no abnormal H_2 increase) during an STT ($n=100$) and during an LTT ($n=109$)

viously (4) The mean value for Δ glucose in the sucrose tolerant group is significantly higher ($p<0.0001$) than in the lactose tolerant group (Table 1)

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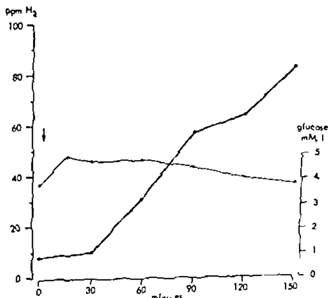


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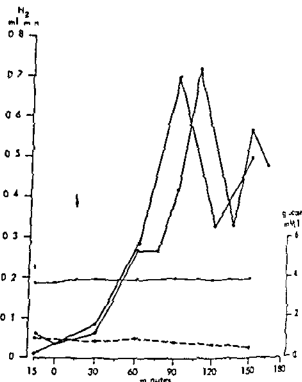


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Acta Pædiatr Scand 69 83–88, 1980

RENAL COMPENSATORY HYPERTROPHY IN CHILDREN WITH UNILATERAL RENAL DISEASE

P WILTON A APERIA O BROBERGER and I WIKSTAD

From the Departments of Paediatrics and Paediatric Radiology Karolinska Institute St Goran s Children s Hospital Stockholm Sweden

ABSTRACT Wilton P, Aperia, A, Broberger O and Wikstad, I (Departments of Pediatrics and Pediatric Radiology, Karolinska Institute St Goran s Children's Hospital, Stockholm Sweden) Renal compensatory hypertrophy in children with unilateral renal disease Acta Paediatr Scand, 69 83 1980 —Kidney parenchymal size was estimated on urograms from 22 children with unilateral vesico-ureteral reflux (VUR) 14 children with bilateral VUR and seven children with unilateral heminephrectomy In the bilateral VUR group one kidney was roentgenologically normal and the other was growth retarded The GFR was estimated in 19 of the children The age of the children was 3–17 years and all

lateral kidney, which was proportional to the parenchymal reduction This compensation was inhibited in the bilateral VUR group There was a positive correlation between the GFR and kidney size

KEY WORDS Renal hypertrophy, vesico-ureteral reflux, kidney size, renal function hemi nephrectomy

When one kidney is removed the remaining kidney will increase its size and function This old observation has led to a series of investiga tions into the nature of compensatory growth and the functional adaptation to changes in renal size Most of the studies have been per formed in animals where the experimental design has been unilateral nephrectomy with or without partial infarction of the contralate ral kidney (3 8 10 16) Knowledge of renal compensatory hypertrophy in man has mainly been derived from studies of kidney donors (5 9 13 15)

Surprisingly little is known about the sig nificance of compensatory growth in unilateral or focal renal disease except that there is some evidence that compensatory hypertro phy can occur in the intact nephrons in chronic pyelonephritis (2 6 12) The aim of the pres ent study was therefore to quantify the hyper trophic capacity of the renal parenchyma in patients having recurrent urinary tract infec tions and kidneys with and without vesico-ureteral reflux as well as in patients who have been subjected to heminephrectomy because of recurrent pyelonephritis

MATERIAL

The material consisted of 43 children 3–17 years of age with recurrent urinary tr

Grade I reflux in the ureter but not extending into the pelvis

Grade II reflux extending into the pelvis without dila tation of the ureter or pelvis

Grade III reflux extending into the pelvis with dilata tion of the ureter or of both the ureter and the pelvis

In group A 17 of 22 children had grade III VUR The

report finding the same high incidence of false flat curves during STT and LTT, 24–33% and 23–30% respectively (6) This may be due to the smaller number of children and absence of H_2 determinations as a reliable indicator for disaccharide malabsorption in that study Our data indicate that the diagnostic value of STT and LTT is different in that secondary sucrase deficiency is not likely to be detected by the STT, even with the help of H_2 breath analysis Theoretically, doubling the sucrose challenge to 4 g/kg might be helpful but since this load is unphysiological we did not study it We therefore recommend that the STT, in contrast to the LTT, should not be used as a routine procedure for the detection of secondary disaccharide malabsorption in children and infants It may only be useful in children thought to have primary sucrase isomaltase deficiency

ACKNOWLEDGEMENTS

We thank Ludy van Winsen for help with the tests Drs J G M Huymans A Klippel and W Rietveld for technical assistance the Children s Department of the Free University Hospital Amsterdam for financial support Dr H M Berger for help with the manuscript

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Submitted May 18 1979
Accepted July 15 1979

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Acta Pædiatr Scand 69 83–88 1980

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KEY WORDS Renal hypertrophy, vesico-ureteral reflux, kidney size, renal function, hemi nephrectomy

When one kidney is removed the remaining kidney will increase its size and function This old observation has led to a series of investigations into the nature of compensatory growth and the functional adaptation to changes in renal size Most of the studies have been per formed in animals where the experimental design has been unilateral nephrectomy with or without partial infarction of the contralateral kidney (3 8 10 16) Knowledge of renal compensatory hypertrophy in man has mainly been derived from studies of kidney donors (5 9 13 15)

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MATERIAL

The material consisted of 43 children 3–17 years of age with recurrent urinary tract infections (UTI) They were divided into three groups (A) 2? children ...

Grade I reflux in the ureter but not extending into the pelvis

Grade II reflux extending into the pelvis without dilatation of ...

Table 1 *Clinical data of patients with unilateral reflux*

Patients	VUR (grade)	Age at urography (years)	Follow up prior to urography (years)	Size of small kidney (% of control)	Size of large kidney (% of control)	No of recurrences during follow up
A B	I	3 3	2 6	33	129	1
U C	II	12 1	6 0	42	119	3
S M	III	6 3	5 5	52	115	3
S J	III	4 6	1 7	53	143	1
L B	III	4 3	1 3	54	120	1
G N	III	3 6	3 5	54	128	2
H B	III	5 7	1 8	61	139	1
P J	II	6 2	5 0	63	92	8
J T	III	6 0	3 3	71	90	2
R D	II	5 5	3 5	72	111	1
R L	III	6 3	1 3	73	80	7
I A	II	11 0	4 0	73	80	9
D I	III	3 1	2 5	73	118	2
A J	III	9 0	4 5	80	100	5
P S	III	4 3	4 0	84	116	4
M L	III	5 3	1 3	88	95	1
L D	III	15 3	8 0	89	105	4
A E	III	5 9	1 0	90	104	1
T O	III	6 3	1 3	90	109	2
S E	III	11 0	3 3	93	107	3
M S	III	6 3	2 3	94	116	2
J B	III	5 5	1 0	97	100	1

group A had grade II VUR and one patient had grade I VUR These five patients and 10 of 17 patients with grade III VUR had one growth retarded kidney Children were excluded who had undergone a urologic operation prior to this investigation had a duplicated pelvis or had shown bilateral reflux on one or more occasions on repeated micturition cystography

All 14 children in group B had bilateral VUR but only one kidney with parenchymal reduction Seven of them had bilateral grade III VUR on each examination The remaining seven did not have grade III VUR into both

age of 8 months to 10 years They all had a unilateral duplicated pelvis The operation was performed two to five years prior to this investigation Only one of them has had a UTI after the operation All had grade II III VUR into the operated pelvis but only two of them had reflux into the other pelvis of the same kidney A further two developed a small VUR post operatively into the operated kidney None had reflux into the non operated kidney

Urine cultures were obtained in most patients every two to three months In addition a urine culture was always taken if there were any symptoms of UTI In each case of significant bacteriuria antimicrobial therapy was given If the incidence of UTI exceeded one/year long term treatment with nitrofuradantoin 1 mg/kg/day was given The height and weight of all patients were within ±2 S D of the mean of normal Swedish children (7)

METHODS

Micturition cystography After bladder catheterization the contrast medium (Isopaque Cysto Nyegaard) was infused into the bladder under low pressure On the first examination anterior and lateral roentgenograms of the bladder and urethra were taken simultaneously during voiding and an anterior roentgenogram of the bladder and kidneys was taken at the end of voiding in order to study

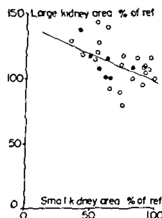


Fig 1 Relationship between the areas of the small and the large kidney in children with unilateral VUR (O) and in heminephrectomized children (●) The solid line represents the regression line for these two groups of patients

Table 2 *Clinical data of heminephrectomized patients*

Patients	Age at urography (years)	Follow up prior to urography (years)	Follow up after hemi nephrectomy (years)	Heminephrec tomized kidney (% of control)	Size of contralateral kidney (% of control)	No of recurrences during follow up
B H	9 9	7 5	4 8	40	138	0
M H	8 0	5 0	3 5	48	117	3
S P	3 8	3 1	3 8	55	108	2
A B	10 5	6 5	4 5	60	101	6
H G	14 9	6 0	5 5	61	115	4
D J	3 5	3 3	2 0	63	100	1
M H	4 2	3 5	3 3	81	108	9

reflux On follow up examinations for reflux one or two roentgenograms were taken of the kidneys and bladder

Kidney size The size of the kidney was measured with a planimeter on urograms and expressed as a per centage of the values found in a control group of children (1) The kidney area was related to body surface Uro graphy was performed in the standard manner reported previously (18)

Renal function In all children with bilateral reflux and in five with unilateral reflux the glomerular filtration rate (GFR) was estimated The standard clearance technique was used which consists of continuous infusion of Inutest 0 001 g/min/kg BW after a prime dose of 0 05 g/kg BW For urine sampling the bladder was catheterized with a double lumen polyethylene catheter enabling continuous suction For unilateral clearance studies external ureteral compression technique was used Complete ureteral occlusion was assessed by highamplifying fluoroscopy The method is described in detail elsewhere (4)

RESULTS

In group A 22 patients were followed 1 0–8 0 years prior to urography Kidney size and the number of recurrences of UTI are shown in Table 1 The smallest kidney was always the reflux kidney and it varied between 33–97% of the size expected for age Of the 22 kidneys with VUR 15 were less than −2 S D i e <85% In nine of these 15 patients the contra lateral kidney was enlarged to more than +2 S D of the expected size The sum of the size of both kidneys in these 15 patients corre sponded to 77–100% of the total kidney paren chyma expected for individuals with two normal kidneys Recurrences of UTI were commoner in the six patients without hyper trophy than in the nine patients with hyper trophy

Seven patients in group C are presented in Table 2 Postoperative urography was per formed 2 0–5 5 years after heminephrectomy The kidney size on the operated side was 40–81% of that expected for age and 100–138% on the non operated side

Fig 1 shows the relationship between the size of both kidneys in patients with unilateral renal disease, i e groups A and C The rela tive size of the smaller kidney is regarded as the independent variable and the size of the larger kidney as the dependent The regression line has the equation $Y=0\ 4323X+140\ 2$ The coefficient by which X is multiplied (−0 4323) has a standard deviation of 0 148 and differs significantly from 0 Extrapolation of the re gression line in Fig 1 to $X=0$ (which means that there is no functional parenchyma in one kidney) gives the value $Y=140\%$ of the size of one kidney in healthy individuals This cor responds to 70% of the size of both kidneys in healthy individuals, and it is in close agree ment with the size of the remnant kidney in nephrectomized patients, which averages 75% of the age expected size of two kidneys in healthy individuals (19)

The data from 14 patients in group B are presented in Table 3 The relationship be tween the small and the large kidney in this group of patients is examined in Fig 2 The regression line is $Y=0\ 0001X+104\ 2$ The coefficient by which X is multiplied has a

Table 3 *Clinical data of patients with bilateral reflux*

Patients	VUR (grade)	Age at urography (years)	Follow up prior to urography (years)	Size of small kidney (% of control)	Size of large kidney (% of control)	No of recurrences during follow up
E N	III III	10 9	7 0	40	109	3
B H	III II	11 5	7 5	44	117	8
G L	III III	4 2	3 9	55	88	5
A H	III II	13 3	4 7	62	88	2
K F	III II	9 9	6 1	71	111	7
A C	III III	7 5	5 2	74	92	9
B T	III III	9 2	6 3	74	94	1
S E	III II	13 2	4 5	77	131	2
E O	III III	9 0	4 7	80	93	5
A J	III III	17 3	15 3	80	117	2
J S	III II	4 3	4 1	81	99	3
G G	III II	9 9	1 7	81	126	2
I L	II II	7 1	1 8	83	102	2
G N	III III	6 0	5 5	84	92	9

standard deviation of 0 285 and is not significantly different from 0

The mean for the smaller kidney is 70 42, with a standard error of 4 03, which is quite close to that observed in groups A+C 68 52 ±3 38 The mean for the larger kidney is 104 21±3 98, which may be compared to 110 60±2 99 in groups A+C

The large kidney in seven of 14 patients in group B was associated with grade III VUR Six of the seven kidneys failed to develop hypertrophy There was no correlation between the number of recurrences of UTI and the size of the kidney in group B

GFR was estimated in five patients in group A and in all patients in group B The relation ship between the kidney area and GFR in all patients with hypertrophied kidney is shown in Fig 3 The relationship is the same as that found in 13 nephrectomized children who have previously been reported from this laboratory (19)

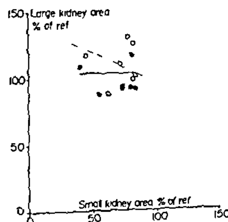


Fig 2 Relationship between the areas of the small and the large kidney in children with grade III bilateral VUR (●) and in children with grade III VUR to one kidney and grade II VUR to the other kidney (O) The solid line represents the regression line for both groups of children The interrupted line is the regression line shown in Fig 1

DISCUSSION

A positive correlation between the degree of loss of renal tissue and the degree of hypertrophy in the remaining renal tissue has previously been shown in rats where 50–80% of the renal tissue was removed (11) When 50% of renal tissue was removed (i e , when unilateral nephrectomy was performed) the size of the remaining kidney finally averaged 70% of the size of two normal kidneys It has now been demonstrated in man that a reduction of the functional parenchyma in one kidney leads to a proportional increase in the size of the contralateral kidney, provided its urinary outflow is normal As a result, the total functional renal parenchyma in unilateral renal dis-

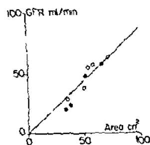


Fig 3 Relationship between kidney area and GFR in children with a hypertrophied kidney (O) represents kidneys from children with unilateral VUR and (O) represents kidneys from children with bilateral VUR The regression line from a group of previously reported 13 nephrectomized children (19) has been extended through origo

ease will always exceed 70% of that found in healthy individuals with two kidneys

In the hypertrophic kidney in patients with unilateral or bilateral vesico ureteral reflux as well as in the remnant kidney, in nephrectomized patients the correlation between glomerular filtration rate and renal size was the same Thus the renal hyperfunction in all of these conditions results from enlargement of renal tissue rather than hemodynamic adaptation This agrees with observations in rats that have been allowed to adapt to ablation of renal tissue for some time (8 17)

All patients in the present study had previous histories of urinary tract infection Thus UTI does not necessarily prevent compensatory hypertrophy in man This is in agreement with previous observations Microdissection studies have shown that there are hypertrophic nephrons among atrophic nephrons in kidneys from humans with chronic kidney disease (14) Bricker et al have performed unilateral nephrectomy in dogs with chronic pyelonephritis and found that the remnant kidney was capable of hypertrophy (6) Micropuncture studies in rats with chronic pyelonephritis show that there are nephrons with reduced single nephron glomerular filtration [illegible]

renal lesion when some nephrons are left intact and are capable of hypertrophy The effect on renal size and function will depend on the degree of pyelonephritic damage The degree of damage can be influenced by, inter alia the number of infections

Selection of patients with bilateral disease was based on the presence of one kidney that was scarred and one kidney with a normal parenchyma The kidney that appeared to be relatively normal but had grade III VUR was usually not capable of hypertrophy Thus, pronounced VUR appears to inhibit hypertrophy This observation deserves more attention from pediatric urologists At present, the main indication for operation of VUR is prevention of pyelonephritis

ACKNOWLEDGMENTS

This study was supported by grants from The Swedish Medical Research Association (B79 19X 02049 13A) and from the research funds of Karolinska Institute

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Table 3. *Clinical data of patients with bilateral reflux*

Patients	VUR (grade)	Age at urography (years)	Follow up prior to urography (years)	Size of small kidney (% of control)	Size of large kidney (% of control)	No of recurrences during follow up
E N	III III	10 9	7 0	40	109	3
B H	III II	11 5	7 5	44	117	8
G L	III III	4 2	3 9	55	88	5
A H	III II	13 3	4 7	62	88	2
K E	III II	9 9	6 3	71	111	7
A C	III III	7 5	5 2	74	92	9
B T	III III	9 2	6 3	74	94	1
S E	III II	13 2	4 5	77	131	2
E O	III III	9 0	4 7	80	93	5
A J	III III	17 3	15 3	80	117	2
J S	III II	4 3	4 1	81	99	3
G G	III II	9 9	1 7	81	126	2
I L	II II	7 1	1 8	83	102	2
G N	III III	6 0	5 5	84	92	9

standard deviation of 0 285 and is not significantly different from 0

The mean for the smaller kidney is 70 42, with a standard error of 4 03, which is quite close to that observed in groups A+C 68 52 ±3 38 The mean for the larger kidney is 104 21±3 98, which may be compared to 110 60±2 99 in groups A+C

The large kidney in seven of 14 patients in group B was associated with grade III VUR Six of the seven kidneys failed to develop hypertrophy There was no correlation between the number of recurrences of UTI and the size of the kidney in group B

GFR was estimated in five patients in group A and in all patients *in group B* The relation ship between the kidney area and GFR in all patients with hypertrophied kidney is shown in Fig 3 The relationship is the same as that found in 13 nephrectomized children who have previously been reported from this laboratory (19)

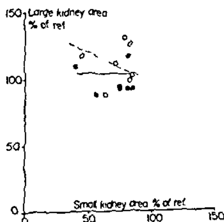


Fig 2 Relationship between the areas of the small and the large kidney in children with grade III bilateral VUR (●) and in children with grade III VUR to one kidney and grade II VUR to the other kidney (O) The solid line represents *the regression* line for both groups of children The interrupted line is the regression line shown in Fig 1

DISCUSSION

A positive correlation between the degree of loss of renal tissue and the degree of hypertrophy in the remaining renal tissue has previously been shown in rats where 50–80% of the renal tissue was removed (11) When 50% of renal tissue was removed (i e , when unilateral nephrectomy was performed), the size of the remaining kidney finally averaged 70% of the size of two normal kidneys *It has now* been demonstrated in man that a reduction of the functional parenchyma in one kidney leads to a proportional increase in the size of the contralateral kidney, provided its urinary outflow is normal As a result, the total functional renal parenchyma in unilateral renal dis-

Acta Pædiatr Scand 69 89–91, 1980

RECENT TRENDS IN BREAST-FEEDING IN SOUTHERN FINLAND

MATTI VERKASALO

From the Children s Hospital University of Helsinki Helsinki Finland

ABSTRACT Verkasalo, M (Children's Hospital, University of Helsinki, Finland) Recent trends in breast feeding in southern Finland Acta Paediatr Scand, 69 89, 1980.—A retrospective study on breast feeding (BF) during 1962–77 has been carried out for the province of Uusimaa The study was based on 35815 child health records kept by community health centres Information was entered in 76% of the records, the percentage improving from 52 to 94 during the study period The average duration of BF was observed to decline steadily from 2.3 months in 1962 to 1 7 in 1971, increasing thereafter to 3 6 months by the end of the study A similar pattern of slow decline turning to a sharp rise by 1971 can be seen in the percentage of mothers beginning BF, and various time categories of nursing The observed increase in BF is attributed to changing general attitudes, which are influenced by international trends and campaining by health authorities Social benefits

KEY WORD Breast feeding

Breast-feeding (BF) has undergone a long decline in the industrialised countries from the early part of this century This was demonstrated in Finland by Salmi (6), Hallman et al (3) and Hultin et al (4, 5) During the last few years, however, BF has gained new popularity in some countries (1, 2, 7) As part of a study on cow's milk protein intolerance (11), a retrospective study was carried out on BF trends in southern Finland between 1962 and 1977

METHODS

Data on BF were extracted from the health records of 35815 infants in the Helsinki metropolitan area one urban community and three [illegible]

[illegible] visits of the child The period of BF indicated in the records also includes the time the child was receiving supplemental feeding in addition to breast milk The attendance of infants at the health centres

varied from 93 to 97% and the [illegible]

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RESULTS

Reliable information on the duration of BF was found in 27087 health records, or 76% of the sample In the rest entries were inadequate or lacking In 1962, when the health record forms were first introduced in health centres, the percentage of entries regarding BF was as low as 53, improving to 94 in 1977

The changes in the duration of BF observed are presented in Fig 1 together with corresponding information from two earlier studies In 1971 the mainly declining course begins to turn to a steep rise The change is observed first in 1970–1972 in the group breast-fed less than one month, and is seen even more clearly if the sample is divided into three parts according to the domicile. Thereafter the aver-

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Submitted May 10 1979
Accepted June 26 1979

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Acta Pædiatr Scand 69 89–91, 1980

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METHODS

Data on BF were extracted from the health records of 35815 of
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RESULTS

Reliable information on the duration of BF was found in 27 087 health records, or 76% of the sample In the rest entries were inadequate or lacking In 1962 when the health record forms were first introduced in health centres, the percentage of entries regarding BF was as low as 53, improving to 94 in 1977

The changes in the duration of BF observed are presented in Fig 1 together with cor responding information from two earlier stud ies In 1971 the mainly declining course begins to turn to a steep rise The change is observed first in 1970–1972 in the group breast fed less than one month, and is seen even more clearly if the sample is divided into three parts ac cording to the domicile Thereafter the aver

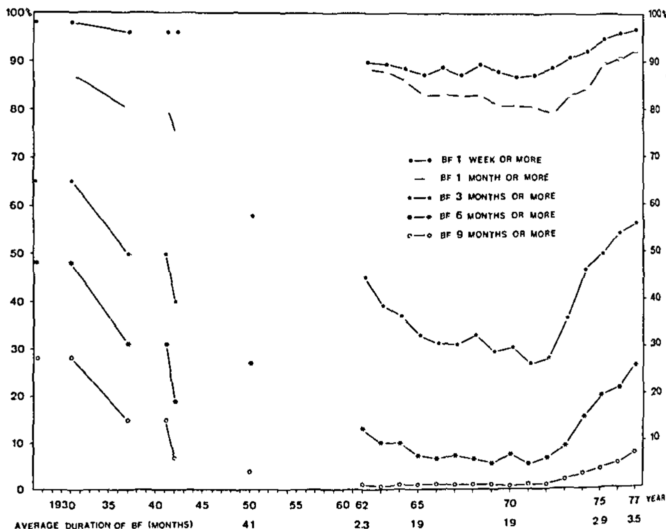


Fig 1 Breast feeding in southern Finland in 1927–1977 Data of 1927–1942 are from Salmi (6) Helsinki and those of 1950 from Hallman et al (3) also from Helsinki Results of this study for 1962–1977 are presented on a stretched time scale

age duration of BF is seen to lengthen considerably, and the percentage of mothers beginning BF is observed to increase from 87 5 to 96 6

DISCUSSION

The results are presented for a retrospective study on BF based on child health centre records from the province of Uusimaa in 1962–1977 The percentage of entries in the records regarding the duration of BF was disappointingly low in the early 60s, which may cause some bias in the results However, the failure in marking down is apparently related to the work load and habits of individual public health workers rather than to any particular attitude toward BF among the mothers

Another factor possibly biasing the results is the change in the population structure due to continuous migration of families each year the population of the province of Uusimaa increased by 3% because of migration (8, 9) According to earlier studies (4, 5), however, the regional differences in BF have diminished, and in 1972 they were almost neglible

Various reasons have been indicated for the observed increase in BF (1–3, 7) Although this study was not designed to resolve the causes underlying the trend, a few suggestions are not out of order

First, in the last decade it has become uni

versally fashionable to turn away from the artificial toward the natural, which must also be a factor influencing general attitudes toward BF (1) Second, in Finland as well as in most other Western countries the health authorities and various societies concerned with the health and feeding of infants have during the last decade intensified their campaign to promote BF Still another factor affecting the BF behaviour of Finnish mothers is changing legislation, which extended the paid maternity leave (with guaranteed return to the previous employment) from two to three months after delivery in 1971, and to seven months in 1974 These extensions must have an effect in a country where approximately 55% of women are employed outside the home (8, 9)

The results of this study are in accordance with earlier reports (4, 5), and in addition indicate that the efforts to enhance BF are bearing fruit and should be continued Furthermore, concentrated campaigns for BF especially in the maternity hospitals promise to improve the figures still more (10)

Interesting to note is the increase in 1971–72 in the proportion of mothers nursing for less than one month This group is thought to include those motivated toward BF, but failing for various reasons As this group is later seen to diminish year by year, one may assume that together with the increasing motivation toward BF also the skill in this basic human function is being transferred more efficiently than only a few years ago

ACKNOWLEDGEMENTS

This study was supported by the Foundation for Chemical Research The Finnish Red Cross Blood Transfusion Service kindly lent its computer capacity for the calculations Help in collecting the data was given by Leena Backstrom, M D , and Jorn Elfving, M D , of the Helsinki Municipal Health Services, and Hannele Monto, R N , of Hyvinkaa Municipal Health Centre

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Submitted June 5 1979
Accepted July 20, 1979

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Acta Pædiatr Scand 69 93–99, 1980

VITAMIN B_{12} IN HUMAN COLOSTRUM AND MILK

Quantitation of the Vitamin and its Binder and the Uptake of Bound Vitamin B_{12} by Intestinal Bacteria

R R SAMSON and D B L McCLELLAND

From the University Department of Therapeutics and Clinical Pharmacology Edinburgh Scotland

ABSTRACT. Samson, R R and McClelland, D B L (University Department of Therapeutics and Clinical Pharmacology, Edinburgh, Scotland) Vitamin B_{12} in human colostrum and milk Quantitation of the vitamin and its binder and the uptake of bound vitamin B_{12} by intestinal bacteria Acta Paediatr Scand, 69 93, 1980 —The concentration of vitamin B_{12} was measured by microbiological assay in 229 samples of normal human colostrum and milk taken at various stages of lactation Colostrum obtained within 48 hours of delivery contains high concentrations of vitamin B_{12} (mean 2431 pg/ml), but within a few days the levels fall to a range similar to the levels in normal serum The vitamin B_{12} binding capacity of 111 samples of colostrum and milk was estimated by gel filtration or charcoal binding Colostrum samples have a mean binding capacity of 72 ng/ml, while the binding capacity in milk is only one third of this value The ability of a range of intestinal bacteria to take up colostrum bound vitamin B_{12}, was assessed All the organisms took up free vitamin B_{12}, but when the vitamin was bound in colostrum, there was little or no uptake even after 24 hours incubation

KEY WORDS Milk vitamin B_{12}, milk vitamin B_{12} binder

There is very little published information about the concentration of vitamin B_{12} in normal human colostrum and milk (2, 8) and most authors quote a single report which contains serious technical flaws (2) There are a number of technical problems in the microbiological assay of vitamin B_{12} in milk which have not been fully dealt with in earlier reports It has been suggested that deoxyribosides (8) and thymidine (19) may stimulate the growth of the assay organism Since Kabata, Ziro & Koda (11) showed that human milk contains ribonucleotides and related metabolites it is necessary to show that these do not interfere with the assay

The binding of vitamin B_{12} in human milk has been described Gregory (8) showed that vitamin B_{12} in untreated milk was not taken up by the assay organism Gullberg (9) measured the binding capacity of a small number of milk samples, but no extensive study of human milk B_{12} binding capacity has been reported

The primary purpose of the present study was to establish the validity of microbiological vitamin B_{12} assay in human colostrum and milk, and to measure vitamin B_{12} concentration and the vitamin B_{12} binding capacity of a substantial number of samples of normal colostrum and milk

In addition, since it has been speculated that the binding of vitamin B_{12} might influence the intestinal flora of the neonate by depriving bacteria of the vitamin (10) we have also investigated the ability of a range of intestinal bacteria to take up vitamin B_{12} which is bound in colostrum Further studies on the biological activity of this vitamin B_{12} binding protein are described in separate papers

MATERIALS AND METHODS

Colostrum and milk

Samples were obtained by manual expression from normal lactating mothers between 1 day and 6 months after deli-

Table 1. *Vitamin B_{12} concentrations in 229 samples of colostrum and milk collected at intervals up to 6 months following parturition*

Sampling time	No of samples	Vitamin B_{12} level (pg/ml) Range	Median
0– 2 days	179	403–13 080	2 022
3– 7 days	19	216–843	375
8–14 days	6	280–427	337
15–21 days	5	118–310	260
22–28 days	4	116–352	282
3– 6 months	16	118–435	262

very None of the donors were receiving treatment with vitamin B_{12} or antibiotics

Assay of vitamin B_{12} levels in human colostrum and milk

Microbiological assay was performed as described by Girdwood (7) using Lactobacillus leichmanni (NCIB 8118) In brief, the method was as follows Protein was precipitated from the samples by steaming for 30 min in acetate buffer, pH 4 6 Following centrifugation, the supernatant was adjusted to pH 6 8 with sodium hydroxide and diluted with distilled water Cyanocobalamin was used as a reference standard in a concentration range of 5 pg to 50 pg/ml Diluted sample or standard was added to 2 ml of double strength medium (Difco vitamin B_{12} assay medium) and the tubes adjusted to 4 ml with distilled water Before adding the assay organism the tubes were sterilized by autoclaving After incubation at 37°C for 18 hours, growth of the assay organism was measured using a Hilger 'Spekker' absorptiometer Each sample was assayed at 4 dilutions, the standards were duplicated All samples were assayed twice on different days

Investigation of factors which may cause over-estimation of vitamin B_{12} in colostrum and milk

Solutions of the bases adenine, cytosine, guanine, thymine and uracil, their 2 deoxynucleosides and their nucleotides, were added separately to aliquots of a pool of colostrum to given concentrations in each case identical to the mean values reported by Kabata et al (11) Other nucleotide derivatives (cyclic-AMP, cytidine diphosphocholine, guanidine diphosphomannose, uridine diphosphoglucuronic acid and uridine diphosphoglucose) were also added at the concentrations described by these workers In a further study of the ability of these metabolites to stimulate the growth of the assay organism, dilutions of each were used in place of the vitamin B_{12} standards in the assay system described

Measurement of the vitamin B_{12} binding capacity of human colostrum and milk

The vitamin B_{12} binding capacity of the samples was assessed by the use of gel filtration to determine the amount of the vitamin bound to high molecular weight constituents Diluted sample (0 5 ml) was mixed at room temperature for 10 min with 1 5 ml of a solution of cobalt 57 vitamin B_{12} containing 40 ng of the vitamin The sample was passed through a column of Sephadex G 200 (2 cm×2 5 cm) at a flow rate of 10 ml per hour using phosphate buffered saline, pH 7 4 Fractions were collected and their radioactivity counted The total activity in th first peak was taken as bound vitamin B_{12} and the activi in the second peak as free vitamin B_{12}

Binding capacity was also assessed by a charcoal ab sorption method Milk or colostrum was mixed with 20 n cobalt-57 vitamin B_{12} The free vitamin was removed fro the sample by mixing for two minutes with a 20% sus pension of buffered activated charcoal powder (BDH After centrifuging, the radioactivity in the supernatar was measured The ability of the charcoal to remove a least 98% of the vitamin B_{12} used in each tube was verific in each assay. All samples were tested in duplicate on tw different days

Bacterial uptake of free and bound vitamin B_{12}

Bacteria were inoculated into media containing added fre vitamin B_{12} or vitamin B_{12} bound to colostrum A singl pool of colostrum-bound vitamin B_{12} was prepared for us in all the experiments Twenty colostrum samples wer pooled and the vitamin B_{12} binding capacity was assesse by gel filtration The total B_{12} binding capacity of the po was calculated to be 2 600 ng A solution containing 9 μC cobalt-57 vitamin B_{12} and non radioactive vitamin B_{12} w added, sufficient to saturate 80% of the available B_{12} bin ing capacity (This degree of saturation was chosen t minimise the chance of free vitamin B_{12} being present After 10 min mixing at room temperature the pool w dialysed against two changes of 5 l of distilled water f 72 h The radioactivity of the pool was measured and was diluted to contain 10 ng vitamin B_{12} per ml The po was stored frozen in aliquots at −30°C

To assess bacterial uptake of vitamin B_{12} a suspensio of each organism was inoculated into 10 ml of mediu containing either 5 ng of colostrum bound cobalt vitamin B_{12} or 5 ng of free vitamin The cultures we incubated for various times at 37°C and centrifuged 2000×g for 30 min Assessment of uptake was made b counting the remaining cobalt 57 activity in the supern tant Different media and culture conditions were us [illegible]

faeces and one Bacteroides (N C T C 10582) 2 cultur of Bifidobacterium bifidus (N C T C 10471 and 1047 one culture of Clostridium sporogenes and one of l leichmanni

RESULTS

Vitamin B_{12} concentrations in colostrum and milk

The results of the vitamin B_{12} assays on 22 samples of colostrum and milk, taken from

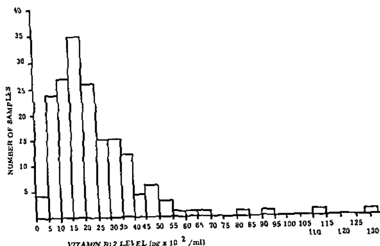


Fig 1 The distribution of vitamin B_{12} concentrations in 179 samples of colostrum collected within 48 hours of parturition

day up to 6 months after delivery are shown in Table 1, and the distribution of concentration in 179 colostrum samples obtained during the first 48 hours is shown in Fig 1 The range of concentrations in the later samples was much narrower

Recovery of vitamin B_{12} added to colostrum

Vitamin B_{12} was added (250 pg/ml of colostrum) to 3 samples The recovery of assayable vitamin B_{12} was 96%, 106% and 108% respectively in the three samples

Growth inhibitors in the test samples

The presence of factors in the test samples which might suppress the growth of L leichmanni was excluded by performing a vitamin B_{12} assay on pooled colostrum over a range of four increasing dilutions Taking the initial result as 100%, levels of 100%, 93% and 93% respectively were found in the dilutions

Stimulation of growth of L leichmanni by substances other than vitamin B_{12} in colostrum

The addition to colostrum of bases, nucleosides nucleotides or nucleotide derivatives as described in methods, did not stimulate growth of the assay organism Indeed the addition of the free bases depressed growth by up to 31% When a range of concentrations of these substances was used to replace vitamin B_{12} in the standard assay system, only the nucleosides could be shown to stimulate growth However, stimulation was only found in concentrations approximating 60 times those which would be attained during the assay of colostrum or milk samples

Vitamin B_{12} binding capacity of colostrum and milk

Nine samples were assayed using both the charcoal binding and gel filtration techniques Binding capacities from 29 4 ng/ml to 120 ng/ml (mean 72 26 ng) were found by gel filtra-

Table 2 *Vitamin B_{12} binding capacity of 111 samples of colostrum and milk collected at various intervals following parturition*

Sampling time	No of samples	Vitamin B_{12} binding capacity (ng/ml)	
		Range	Median
0– 2 days	63	4 8–114 0	75
3– 7 days	17	23 6– 37 8	35 6
8–14 days	7	19 0– 33 2	21 8
15–21 days	7	12 2– 33 8	18 4
22–28 days	7	11 6– 35 8	28 6
3– 6 months	10	3 0– 31 6	19 1

Table 1. *Vitamin B_{12} concentrations in 229 samples of colostrum and milk collected at intervals up to 6 months following parturition*

Sampling time	No of samples	Vitamin B_{12} level (pg/ml) Range	Median
0– 2 days	179	403–13 060	2 022
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Binding capacity was also assessed by a charcoal absorption method. Milk or colostrum was mixed with 20 ng cobalt-57 vitamin B_{12} The free vitamin was removed from the sample by mixing for two minutes with a 20% suspension of buffered activated charcoal powder (BDH) After centrifuging, the radioactivity in the supernatant was measured The ability of the charcoal to remove at least 98% of the vitamin B_{12} used in each tube was verified in each assay All samples were tested in duplicate on two different days.

Bacterial uptake of free and bound vitamin B_{12}

Bacteria were inoculated into media containing added free vitamin B_{12} or vitamin B_{12} bound to colostrum A single pool of colostrum bound vitamin B_{12} was prepared for use in all the experiments Twenty colostrum samples were

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cobalt-57 vitamin B_{12} and non radioactive vitamin B_{12} was added, sufficient to saturate 80% of the available B_{12} binding capacity (This degree of saturation was chosen to

[illegible]

72 h The radioactivity of the pool was measured and it was diluted to contain 10 ng vitamin B_{12} per ml The pool was stored frozen in aliquots at −30°C

To assess bacterial uptake of vitamin B_{12}, a suspension of each organism was inoculated into 10 ml of medium containing either 5 ng of colostrum bound cobalt 57 vitamin B_{12} or 5 ng of free vitamin The cultures were incubated for various times at 37°C and centrifuged at 2000×*g* for 30 min Assessment of uptake was made by counting the remaining cobalt 57 activity in the supernatant Different media and culture conditions were used for the different types of bacteria studied

Five somatic types of E coli were studied 01 05 07, 055 and 0111, 6 cultures of Bacteroides isolated from adult faeces and one Bacteroides (N C T C 10582), 2 cultures of Bifidobacterium bifidus, (N C T C 10471 and 10472), one culture of Clostridium sporogenes, and one of L leichmanii

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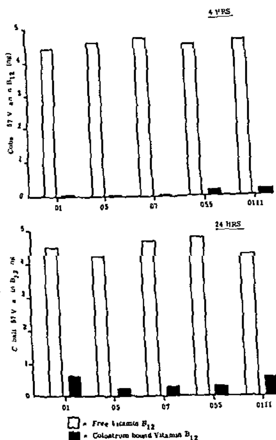


Fig. 3. The uptake of free and colostrum bound cobalt 57 vitamin B_{12} (5 ng) by 5 cultures of E. coli following aerobic incubation at 37°C for 4 hours and 24 hours in Davis and Mingioli medium (3).

Mingioli medium. Following incubation at 37°C for 48 hours, the sample was filtered through Sephadex G200. No free vitamin B_{12} could be identified in the fractions.

DISCUSSION

Vitamin B_{12} concentrations in colostrum and milk

Samples of colostrum obtained within 48 hours of delivery have extremely high vitamin B_{12} concentrations, the median value being about three times the upper limit of normal concentrations in serum. There is a wide scatter in results at this early stage of lactation, as is observed for many other milk proteins (14). By the third week, however, the vitamin B_{12} concentrations fall to a plateau level similar to the normal serum concentration.

There is exceedingly little published information on the vitamin B_{12} content of human colostrum and milk. Standard sources (6, 12) refer to the publication of Collins et al. (2). This paper described observations on very small numbers of samples, moreover the methods used must be considered unsatisfactory in several respects. The vitamin B_{12} measurements were made on whole milk which had not been sterilised. In this system, vitamin B_{12} would not be taken up by the assay organism and could not be measured (8, and the present observations). The growth of the assay organism was measured by acidimetric titration, and it therefore seems likely that the results which were interpreted as growth of the assay organism were in fact due to the growth of contaminants and unrelated to vitamin B_{12} levels.

The only recent publication reporting human milk vitamin B_{12} levels (4) gives the results on 5 large pools of mature milk, all of which had vitamin B_{12} levels of 100 pg/ml. It is surprising that the pools had identical vitamin B_{12} levels since there were appreciable variations in the other constituents measured. The stated B_{12} concentration is substantially lower than the median value found for mature milk in the present study (260 pg/ml).

Technical factors affecting microbiological assay of vitamin B_{12} in colostrum and milk

It has been reported by Coates (1) that deoxyribosides can act as growth factors for lactobacilli, although they are about 1 000 fold less potent than vitamin B_{12}. Kabata et al. (11) showed that human milk contains a wide range of nucleotides. For this reason the influence of nucleotides and related metabolites on the growth of the assay organism was investigated by addition both to colostrum and to the assay medium of the metabolites described in the methods section. None of these substances stimulated growth of the assay organism. It

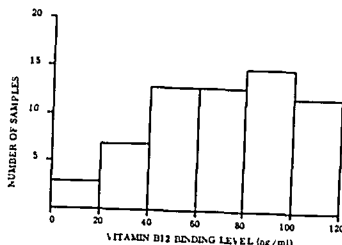


Fig 2 The distribution of vitamin B_{12} binding capacity in 63 samples of colostrum collected within 48 hours of parturition

tion and from 30 to 114 ng/ml (mean 72 32 ng) using the charcoal method, (r=0 95) For all subsequent samples the charcoal technique was used

The vitamin B_{12} binding capacity of 111 samples of colostrum and milk taken at various stages of lactation is shown in Table 2 As the range of values was extremely wide in the samples collected during the first 48 hours of lactation, the distribution of results in these samples is also shown in Fig 2

The rate at which vitamin B_{12} is bound by colostrum was assessed using the charcoal method Aliquots of a pool of colostrum were mixed with 20 ng cobalt-57 vitamin B_{12} and charcoal was added after incubation periods of 1 to 30 min. Following centrifugation the a vity in the supernatant indicated that bind was complete within 1 minute (13 6 ng bour as no significant increase was detected up 30 min (14 4 ng bound) The stability of t binding activity on storage was assessed 10 samples before and after one month −30°C The initial range was 7 1 to 17 8 binding/ml (mean 13 ng/ml) and followi storage 7 to 20 6 ng binding/ml (mean 13 ng/ml)

The uptake of free and colostrum bound vitamin B_{12} by intestinal bacteria

Fig 3 summarises the findings with 5 E co strains which were incubated for four hour and for twenty four hours After four hour there was little uptake of bound vitamin b any organism After 24 hours, although th uptake was slightly greater, it remained ver much less than the uptake of free vitamin

Table 3 shows the uptake of the free an bound vitamin by the other cultures tested With each organism the uptake of the fre vitamin greatly exceeded that of the bound

Release of bound vitamin B_{12} during incubation

To check if vitamin B_{12} could dissociate from the colostrum binding protein during the incu bation periods used 30 ng of colostrum bound vitamin B_{12} was added to 7 ml of Davis and

Table 3 *The uptake of free and colostrum bound cobalt-57 vitamin B_{12} (5 ng) by various test bacteria*

	No of cultures tested	Free vitamin B_{12} (5 ng) Range	Mean	Colostrum bound vitamin B_{12} (5 ng) Range	Mean
Bacteroides[a]	7	3 4 –4 8	4 3	0 5–1 3	1 0
Bifidobacteria[b]	2	4 65–4 75	4 7	0 5–0 7	0 6
Cl sporogenes[a]	1	–	2 5	–	0 2
L leichmanii[c]	1	–	4 5	–	0

[a]
[b]
[c]

The relevance of the vitamin B_{12} binding activity to the wellbeing of the neonate remains uncertain at present In this study of a large number of samples we have shown that substantial quantities of both vitamin B_{12} and vitamin B_{12} binding capacity are normal constituents of human colostrum and milk Bovine milk has very little vitamin B_{12} binding capacity (5) but contains more vitamin B_{12} than human milk (8), and infant feeds based on cow s milk also contain 5–10 times more vitamin B_{12} than mature human milk (13) The importance of this striking difference between two forms of infant feed requires further investigation

ACKNOWLEDGEMENTS

The skilful assistance of the nursing staff of the Simpson Memorial Maternity Pavilion in providing the many samples for this study is gratefully acknowledged

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Submitted Feb 11 1979
Accepted June 15 1979

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is therefore considered highly unlikely that the presence in colostrum of any of these nucleotides or related substances could interfere with the vitamin B_{12} assay used

The possibility that a growth inhibitor in colostrum could affect the assay organism was also considered to be excluded by the evidence that the measured vitamin B_{12} concentrations were constant over a range of sample dilutions If an inhibitor were present its influence would be expected to diminish at higher sample dilution

Vitamin B_{12} binding capacity in colostrum and milk

The vitamin B_{12} binding capacity changes in the same way as the vitamin B_{12} concentration with very high values in the first few days followed by a rapid fall to a more uniform level The binding capacities found are similar to those reported by Gullberg (9) in a study of 5 samples

This pattern of concentration change during early lactation is very similar to that observed with secretory IgA and suggests that the binding protein is produced locally in the breast, and probably accumulates before lactation becomes established During the first few days of lactation although the volume of colostrum and milk produced may be very small, the very high concentration of the binding protein permits a large total daily intake by the infant Later in lactation, despite the intake of a larger milk volume, the total intake of the binding protein may be less, this pattern of events has been shown previously for a number of milk proteins (14)

The B_{12} binding capacity of colostrum and milk is always highly unsaturated (mean saturation in colostrum 3 6% and in milk 1 4%) and the binding of vitamin B_{12} is avid The binding protein would therefore seem to be an effective mechanism for accumulating vitamin B_{12} for transport from mother to infant It is not clear, however, whether the vitamin B_{12} bound in colostrum and milk is available for [illegible] infant The B binder complex is resistant to digestion by pepsin but less resistant to trypsin (17), and it appears to have no intrinsic factor-like absorptive functions (18)

The effect of binding by colostrum and milk on the uptake of vitamin B_{12} by intestinal bacteria

It was shown by Gregory (8) that L leichmanii did not grow in an ultrafiltrate of human milk indicating that growth factors for the organism (including vitamin B_{12}) were bound to a non dialysable component Ford (5) has shown that the milk of sow and goat has the capacity to bind vitamin B_{12} and make it unavailable to a range of bacteria, and that B_{12} bound by human milk was not taken up by E coli 0101K There have, however, been no extensive studies of the ability of human colostrum and milk to make vitamin B_{12} unavailable to a range of bacteria likely to be found in the normal intestine The present data show that although all the organisms tested would take up free vitamin B_{12}, none took up the vitamin when it was bound by colostrum, even after prolonged incubation

It has been speculated that this ability to make vitamin B_{12} unavailable to bacteria could be a factor influencing the growth of organisms in the neonatal intestine (10, 5) but there has been no direct evidence for this suggestion We have recently shown that the B_{12} binding protein in colostrum inhibits the growth of E coli of known vitamin B_{12} dependence, (16) but has no effect in the growth of strains which are not B_{12} dependent All the organisms examined in the present study were capable of growth in the absence of vitamin B_{12} Thus if the vitamin B_{12} binding protein is to have an influence on the bacterial ecology of the infant s intestine it is necessary to postulate that this effect will occur under particular environmental conditions perhaps when there is a lack of metabolites which can replace the vitamin or in the presence of other antibacterial factors which may act in synergy with the effects of vitamin B_{12} depletion

Acta Pædiatr Scand 69 101–103 1980

TOLAZOLINE AND DOPAMINE THERAPY IN NEONATAL HYPOXIA AND PULMONARY VASOSPASM

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ABSTRACT Hegyi, T and Hiatt, I M (Division of Perinatology, Monmouth Medical Center and Department of Pediatrics, the Hahnemann Medical College, Philadelphia, USA) Tolazoline and dopamine therapy in neonatal hypoxia and pulmonary vasospasm Acta Paediatr Scand, 69 101, 1980.—Severe hypoxia unresponsive to maximum ventilatory support occurs both in idiopathic respiratory distress syndrome and meconium aspiration We recently encountered a 980 g female infant with respiratory distress syndrome and 3300 g female infant with meconium aspiration and persistant fetal circulation whose clinical course necessitated the use of tolazoline and dopamine to reduce pulmonary and to stabilize systemic pressures The infant with respiratory distress syndrome responded with a $P_{a_{O_2}}$ increase of 2 7 kPa while the infant with persistant fetal circulation and meconium aspiration showed a 51 6 kPa rise Combined pharmacologic therapy may have a role in improving oxygenation status in severely hypoxemic infants receiving maximum support

KEY WORDS Tolazoline, dopamine, hypoxia respiratory distress syndrome, meconium aspiration syndrome

Pulmonary hypertension has been suggested to be an important contributing factor in the development of severe hypoxemia in both idiopathic respiratory distress syndrome (2) and perinatal aspiration syndromes (5) Hypoxia and acidosis, common to both of these disease entities have been found to be potent stimuli to pulmonary vasoconstriction

Attempts to alleviate pulmonary hypoperfusion have included rapid correction of hypoxia and acidosis but have recently been focused on the reversal of pulmonary hypertension with pharmacologic agents such as tolazoline (3 7) whose major side effects have included systemic hypotension Theoretically, a more optimal approach would include maintenance of normal systemic blood pressure in addition to the reduction of pulmonary pressure providing for the maximum reversal of the pulmonary to systemic gradient

We recently encountered two infants suffering from progressive respiratory failure unresponsive to conventional ventilatory therapy The following case reports describe the simultaneous use of tolazoline and dopamine in a premature infant with severe hyaline membrane disease and in a post term infant with meconium aspiration syndrome and persistent fetal circulation

CASE REPORTS

A white female infant weighing 980 g was delivered to a 20-year-old woman after 28 weeks of gestation The pregnancy was complicated by hyperthyroidism that was treated with propylthiouracil Labor was complicated by vaginal bleeding and the infant was delivered by Caesarian section Apgar scores at one and five minutes were 1 and 8

On admission to the newborn intensive care unit physical examination revealed a 28 week infant in severe respiratory distress Chest X ray was consistent with severe respiratory distress syndrome Clinical evaluation chest X ray and EKG results ruled out the existence of congenital heart disease At two hours of age arterial blood gas tensions in an F_iO_2 of 0 95 were as follows pH 7 00 $P_{a_{CO_2}}$ 10 9 kPa and $P_{a_{O_2}}$ 8 6 kPa. Therapy with mechanical ventilation was instituted reaching the following level of support at 5 hours F_iO_2-0 95 PAP-60 cm H_2O IMV-40/min and PEEP 6 cm H_2O At

duce the pulmonic to systemic pressure gradient The improvement of blood pressure was accompanied by a 2 9 kPa increase in P_{aO_2} and a 2 7 kPa reduction in P_{aCO_2} While the P_{aO_2} increase is small, the P_{aCO_2} decrease suggests a considerable improvement in the $AaDCO_2$ gradient (1 14)

The response of our patient with meconium aspiration to the treatment regime was dramatic as P_{aO_2} rose 51 6 kPa and P_{aCO_2} decreased 2 3 kPa The magnitude of the P_{aO_2} change confirms the increased responsiveness of the pulmonary vascular bed in the term or post term infant Whether this is due to the absolute increase in muscle mass in the small pulmonary arteries (9) or to an increase in the number of vasoactive vessels in the pulmonary vascular bed (10) is still under question What has been confirmed by Fox et al (5) is the existence of variable degrees of right to left shunting in infants with meconium aspiration syndrome The effectiveness of tolazoline dopamine therapy is shown in the reduction of our patient s requirements from 90% to 21% inspired oxygen concentration in 10 hours

The decision to administer tolazoline dopamine to these two severely hypoxic infants was based on three observations 1) The high mortality observed in infants with malignant respiratory distress syndrome and massive meconium aspiration syndrome 2) Our previous experience with tolazoline caused hypotension in similar cases 3) The theoretical advantage in stabilizing systemic while simultaneously reducing pulmonic pressure

While progressive hypoxemia was reversed in both infants the contribution of correcting hypoxia acidosis hypoglycemia as well as the role of high pressure ventilation in reducing pulmonary resistance cannot be determined in this clinical study

The pharmacologic therapy of pulmonary hypertension may be an important adjunct in the treatment of severe hypoxemia in respiratory distress syndrome and in persistent fetal circulation of meconium aspiration syndrome However further work is necessary to clarify not only the pharmacology of these agents but also their pulmonary and hemodynamic effects

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Submitted Jan 29 1979
Accepted Aug 1 1979

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this time with the pH 7 07, P_{aCO_2} 1 5 kPa tolazoline 0 5 mg/kg was infused over five minutes via scalp vein followed by a continuous infusion of 0 5 mg/kg/hour Although P_{aO_2} increased to 6 7 kPa and P_{aCO_2} decreased to 7 7 kPa systolic blood pressure dropped from 35 to 25 mmHg In view of this hypotension and a decreasing P_{aO_2} to 4 5 kPa dopamine 5 μg/kg/min was added to the therapy The results of this maneuver were as follows systolic blood pressure rose to 38–42 mmHg P_{aO_2} rose to 7 4–10 0 kPa and P_{aCO_2} decreased to 4 1–4 8 kPa However, despite continued improvement in respiratory status the infant suffered an intraventricular hemorrhage and died at 27 hours of age

A 3300 g white female infant born to a 32 year-old woman at 44 weeks gestation Pregnancy and labor were uncomplicated however, thick meconium was noted at delivery that resulted in a moderately depressed infant requiring endotracheal suctioning as well as resuscitation Apgar scores were 3 and 6 at one and five minutes respectively

At 1 hour of age the arterial blood gas in F_iO_2 of 0 28 showed a pH of 7 17, P_{aCO_2} 5 5 kPa and P_{aO_2} of 8 8 kPa At 5 hours of age a blood glucose less than 25 mg/dl was documented and treated with rapid intravenous infusion of 25% glucose At 6 hours generalized tonic seizure activity was noted and treated with intravenous phenobarbital At 9 hours of age the infant was transported to Monmouth Medical Center

On admission to the intensive care unit, physical examination showed a post term meconium stained female infant in severe respiratory distress Chest X ray revealed hyperaerated lung fields and patchy infiltrates findings consistent with meconium aspiration syndrome Further evaluation with EKG and chest X ray denied the presence of heart disease The infant required assisted ventilation for post asphyxial seizures and apnea Her condition was stable for approximately 6 hours on 40% ambient oxygen concentration when she suffered a tension pneumothorax and severe hypoxemia By 14 hours of age the P_{aO_2} was 5 2 kPa in an F_iO_2 of 0 85 and at 18 hours of age the P_{aO_2} was 2 5 kPa (measured both in the umbilical artery and right brachial artery) in an F_iO_2 of 0 9

At this time with the systolic blood pressure in the range of 50–60 mmHg the decision was made to administer dopamine and tolazoline simultaneously Due to an error the dose of tolazoline given was 5 mg/kg instead of the intended 0 5 mg/kg Systolic blood pressure dropped to 40–55 mmHg but arterial P_{aO_2} rose to 54 3 kPa P_{aCO_2} prior to tolazoline was 5 1–5 6 kPa and after tolazoline it was in the range of 2 9–4 kPa Dopamine was continued for the next 12 hours with blood pressure remaining in the 50–80 mmHg range The infant s respiratory status during this rapidly improved By 10 hours post tolazoline infusion mechanical ventilation and additional oxygen administration were discontinued

DISCUSSION

Respiratory distress syndrome and meconium aspiration syndrome are accompanied by increased pulmonary vascular resistance and reduced lung perfusion (2, 5, 8, 13) The recognition of the contribution of pulmonary hypoperfusion to the pathogenesis of both disorders has led several authors to suggest that therapy for severe respiratory distress syndrome and meconium aspiration syndrome may require the amelioration of pulmonary hypertension (2, 9, 12)

Tolazoline is an alpha adrenergic blocking agent that has been used to improve pulmonary perfusion (7) Pulmonary vasoconstrictor agents such as hypoxia and acidosis have specific alpha adrenergic activity and exert their effects through alpha adrenergic receptors in the lung (11) Pharmacologic blockade of these receptors can reduce vasoconstriction to 20% of control values and theoretically should always be effective in reducing or reversing right to left gradient (11) However, tolazoline also exerts a direct nonadrenergic relaxant effect on vascular smooth muscle that can lead to systemic hypotension (7) The consequence of reduced systemic blood pressure and low peripheral perfusion include the re-establishment of right to left shunting as well as increased anaerobic metabolism The resulting metabolic acidosis from hypoperfusion can lead to adverse effects on the pulmonary vascular bed and further compromise pulmonary perfusion Therefore by reducing both pulmonary and vascular resistance, tolazoline may exert variable effects on the pressure gradient between these two circulations The lack of uniform results with tolazoline during clinical trials has suggested the above hypothesis to have some merit (6)

Although systemic hypotension was not a major problem in a recent report on tolazoline (6) it became a serious one in our premature infant with respiratory distress syndrome Because of falling P_{aO_2} we did not wish to discontinue tolazoline since it had shown transient beneficial effects By increasing systemic blood pressure with dopamine (4) while reducing pulmonary pressure we hoped to re

Acta Pædiatr Scand 69 105–108, 1980

PERSISTENT LEFT SUPERIOR VENA CAVA

Incidence associated Congenital Heart Defects and Frontal Plane P wave Axis in a Paediatric Population with Congenital Heart Disease

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ABSTRACT Bjerregaard, P and Laursen H B (University Department of Cardiology, Aarhus Kommunehospital, Aarhus, Denmark) Persistent left superior vena cava Incidence, associated heart defects and frontal plane P wave axis in a paediatric population with congenital heart disease Acta Paediatr Scand, 69 105, 1980.—Among 3671 patients aged 0–15 years with congenital heart disease diagnosed by cardiac catheterization and/or autopsy, 49 or 1 3% had a persistent left superior vena cava (PLSBC) The distribution of PLSVC

mere chance relationship Abnormal frontal plane P wave suggesting ectopic pacemaker activity was found in 35% of the patients with PLSVC The potential importance of PLSVC for the surgical risk during operation for congenital heart disease and for instability of the cardiac rhythm is emphasized

KEY WORDS Congenital heart disease, persistent left superior vena cava, frontal plane P wave axis

Several authors (2 6 16) have reviewed the relevant anatomy and embryology of persistent left superior vena cava (PLSVC)

This report is the first to give the incidence of PLSVC in a large unselected paediatric population with congenital heart disease diagnosed by cardiac catheterization and/or autopsy

The association between PLSVC and other congenital heart defects is described, and owing to previous reports emphasizing a frequent association between PLSVC and abnormal cardiac rhythms (7, 13, 14), is the frontal plane P wave axis in the ECG analysed

MATERIALS AND METHODS

In order to obtain complete information of the incidence of various types of congenital heart disease in Denmark in the period 1963–73 one of us reviewed the medical records of all children aged 0–15 years who were admitted to either a paediatric or a cardiological department of a Danish hospital in this period and diagnosed as having a congenital heart disease (12) All death certificates for children who died in Denmark with a congenital heart disease in this particular period were also reviewed

Congenital heart disease was found in 5249 children which gives an incidence in Denmark among children aged

cannot be made with certainty by clinical examination alone the patients of the present study were selected from the 3671 in whom the diagnoses were based on cardiac catheterization and/or autopsy

In all the patients with PLSVC associated congenital heart defects were noted and the first available 9-lead ECG analysed The polarity of the P waves in leads I II III and V_4 were determined in order to describe the axis of the frontal plane P wave axes were grouped as suggested by Momma & Linde (13)

RESULTS

A total of 49 patients were found with PLSVC giving an incidence of 1 3% among children aged 0–15 years with congenital heart disease Female to male ratio was 2/3 The diagnosis was made by cardiac catheterization in 23

Acta Pædiatr Scand 69, 105–108, 1980

PERSISTENT LEFT SUPERIOR VENA CAVA

Incidence, associated Congenital Heart Defects and Frontal Plane P-wave Axis in a Paediatric Population with Congenital Heart Disease

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ABSTRACT. Bjerregaard, P. and Laursen, H B. (University Department of Cardiology, Aarhus Kommunehospital, Aarhus, Denmark). Persistent left superior vena cava. Incidence, associated heart defects and frontal plane P-wave axis in a paediatric population with con-

among patients with various types of congenital heart disease did not suggest any causal relationship except in patients with pulmonary atresia and in patients with anomalous pulmonary venous connection, in whom PLSVC was found more frequently than expected by a mere chance relationship Abnormal frontal plane P wave suggesting ectopic pacemaker activity was found in 35% of the patients with PLSVC The potential importance of PLSVC for the surgical risk during operation for congenital heart disease and for instability of the *cardiac rhythm is emphasized*

KEY WORDS: Congenital heart disease, persistent left superior vena cava, frontal plane P wave axis

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MATERIALS AND METHODS

incidence Denmark ... medical ... admitted ... tment of a ... diagnosed as having a congenital heart disease (12) All death certificates for children who died in Denmark with a congenital heart disease in this particular period were also reviewed

Congenital heart disease was found in 5249 children, which gives an incidence in Denmark among children aged 0–15 years of 6 1 per thousand

The diagnosis of a congenital heart disease was based on clinical examination alone in 1578 children whereas

alone, the patients of the present study were selected from the 3671 in whom the diagnoses were based on cardiac catheterization and/or autopsy

In all the patients with PLSVC, associated congenital heart defects were noted, and the first available 9-lead ECG analysed The polarity of the P waves in leads I, II, III and V_4 were determined in order to describe the axis of the frontal plane P wave axes were grouped as suggested by Momma & Linde (13)

RESULTS

A total of 49 patients were found with PLSVC giving an incidence of 1.3% among children aged 0–15 years with congenital heart disease. Female to male ratio was 2/3. The diagnosis was made by cardiac catheterization in 23

Table 1 *Frequency of persistent left superior vena cava according to the main diagnosis in 3761 paediatric patients with congenital heart disease*

Main diagnosis	No of patients	No of patients with PLSVC	Frequency of PLSVC (%)
Ventricular septal defect	720	8	1.0
Persistent ductus arteriosus	479	0	0.0
Atrial septal defect	387	4	1.0
Coarctation of the aorta	322	4	1.2
Tetralogy of Fallot	296	6	2.0
Transposition of the great arteries	253	3	1.2
Pulmonary stenosis	237	1	0.4
Underdeveloped left ventricle	159	4	2.5
Common atrioventricular canal	122	3	2.5
Fibroelastosis	121	0	0.0
Aortic stenosis	116	1	0.9
Cor univentriculare	77	1	1.3
Truncus arteriosus	75	0	0.0
Tricuspid atresia	45	2	4.5
Anomalous pulmonary venous connection	41	4	10.0
Pulmonary atresia	33	2	6.0
Ebstein's anomaly	15	0	0.0
Other cardiac defects	259	2	0.8
Isolated PLSVC	4		
Total	3 761	49	1.3

patients and by autopsy in 12 patients. In 14 patients both cardiac catheterization and autopsy were performed.

The PLSVC was draining into the right atrium via the coronary sinus in 42 patients. In 7 patients it entered the left atrium. A patent right superior vena cava was present in all patients. A PLSVC was found as an isolated anomaly in 4 patients (table 1). In 40% of the patients with PLSVC an atrial septal defect was present, and a frequent association was likewise found between PLSVC and ventricular septal defect, coarctation of the aorta, tetralogy of Fallot, transposition of the great arteries and pulmonary stenosis. PLSVC was never found in patients in whom the main diagnosis was patent ductus arteriosus, fibroelastosis, truncus arteriosus or Ebstein's anomaly. In contrast to this, anomalous pulmonary venous connection and pulmonary atresia were seen more often in association with PLSVC than a mere chance relationship would have provided.

Analysis of the frontal plane P-wave axis was possible in 37 patients. In 12 patients no ECGs were available mainly because these patients had died immediately on birth. A total of 22 patients had an intermediate P wave axis suggesting sinus rhythm. Two patients had right axis deviation of the frontal plane P-wave and 13 had left axis deviation compatible with a diagnosis of coronary sinus rhythm. The ECGs used for analysis had all regular rhythms and normal P-R intervals.

DISCUSSION

PLSVC is usually a harmless phenomenon but can be of importance to the patient. Usually is it without haemodynamic significance, but draining into the left atrium may cause central cyanosis. Its presence can make cardiac catheterization impossible or at least very difficult from the left arm, and attempts to overcome an obstacle at the level of the anastomosis between the left subclavian vein and the jugular vein have led to a fatal outcome in at least 2 patients reported in the lite-

rature (8, 15) An undiagnosed PLSVC may present special problems when venous cannulation is performed in preparation for extracorporeal circulation in the course of cardiac surgery (3, 4)

PLSVC is one of the most frequent anomalies of the venous system (6) Keith et al (11) found this anomaly in one per 350 autopsies giving an incidence of 0 3% which is in good agreement with Steinberg et al (17), who estimated that PLSVC was present in 0 5% of the population A higher incidence has been found in patients with congenital heart disease Campbell & Deuchar (2) found this anomaly in 3% of 1500 patients with congenital heart disease and Fraser et al (5) found an incidence of 4 3% among 703 patients Our finding of an incidence of 1 3% of PLSVC among 3761 paediatric patients with congenital heart disease is somewhat less than what has previously been reported, but the ages and selection of the patients have not been identical in the various series

Gensini et al (6) found a high coincidence of anomalous pulmonary venous connection and atrial septal defect in patients with PLSVC In only 2 out of 37 patients was PLSVC found as an isolated anomaly Among 30 patients with PLSVC, Fraser et al (5) found 9 patients with atrial septal defect, 7 patients with tetralogy of Fallot and 6 patients with anomalous pulmonary venous connection One patient had no associated congenital heart disease Momma & Linde (13) had 26 patients with PLSVC Ten patients had atrial septal defect and 8 patients tetralogy of Fallot Three patients had anomalous pulmonary venous connection and in one patient there were no associated congenital heart disease Our results confirm the frequent association between PLSVC and atrial septal defect Among our 49 patients there were 19 with atrial septal defect, which means that this anomaly is found in approximately 40% of patients with PLSVC encountered during cardiac catheterization or at autopsy However, when patients with isolated atrial septal defect were considered, the association with PLSVC was not greater than expected by a mere chance relationship (Table 1) The high coincidence of atrial septal defect and PLSVC among patients with congenital heart defects is therefore most likely due to the overall high incidence of atrial septal defect Only in patients with anomalous pulmonary venous connection and patients with pulmonary atresia was a higher association with PLSVC than expected by a mere chance relationship found The unexpected high incidence of associated defects in the pulmonary veins and in the systemic veins is, however, well understood when one considers the fact that the embryological evolution of the pulmonary venous system takes place simultaneously with the development of the systemic venous return (6) In order not to miss a simultaneous occurrence of anomalous pulmonary venous connection and PLSVC several blood samples should be drawn at various sites between the right atrium and the subclavian vein during cardiac catheterization

Left axis deviation of the frontal plane P wave associated with PLSVC was initially reported by Hancock in 1964 (7) as inverted P waves in lead III It was present in 14 of 20 patients with PLSVC associated with other congenital heart defects Momma & Linde (13) found left axis of P-waves between +15 and −29 degrees in 9 patients out of 26 with PVLSC associated with other congenital heart defects These two studies thus suggested a frequency of left axis deviation of the P-wave in patients with PLSVC of approximately 50% In contrast to this Burnett & Taylor (1) found an incidence of left axis of the P wave in normal children of 10% and Hancock (7) found only 5% among 62 patients with atrial septal defect

Our study has confirmed the relatively high incidence of left axis deviation of the frontal plane P-wave associated with PLSVC in a paediatric population with congenital heart disease Among 37 patients with PLSVC associated with other congenital heart defects 13 patients or 35% had this anomaly

Table 1. *Frequency of persistent left superior vena cava according to the main diagnosis in 3761 paediatric patients with congenital heart disease*

Main diagnosis	No of patients	No of patients with PLSVC	Frequency of PLSVC (%)
Ventricular septal defect	720	8	1 0
Persistent ductus arteriosus	479	0	0 0
Atrial septal defect	387	4	1 0
Coarctation of the aorta	322	4	1 2
Tetralogy of Fallot	296	6	2 0
Transposition of the great arteries	253	3	1 2
Pulmonary stenosis	237	1	0 4
Underdeveloped left ventricle	159	4	2 5
Common atrioventricular canal	122	3	2 5
Fibroelastosis	121	0	0 0
Aortic stenosis	116	1	0 9
Cor univentriculare	77	1	1 3
Truncus arteriosus	75	0	0 0
Tricuspid atresia	45	2	4 5
Anomalous pulmonary venous connection	41	4	10 0
Pulmonary atresia	33	2	6 0
Ebstein s anomaly	15	0	0 0
Other cardiac defects	259	2	0 8
Isolated PLSVC	4		
Total	3 761	49	1 3

patients and by autopsy in 12 patients In 14 patients both cardiac catheterization and autopsy were performed

The PLSVC was draining into the right atrium via the coronary sinus in 42 patients In 7 patients it entered the left atrium A patent right superior vena cava was present in all patients A PLSVC was found as an isolated anomaly in 4 patients (table 1) In 40% of the patients with PLSVC an atrial septal defect was present, and a frequent association was likewise found between PLSVC and ventricular septal defect, coarctation of the aorta, tetralogy of Fallot, transposition of the great arteries and pulmonary stenosis PLSVC was never found in patients in whom the main diagnosis was patent ductus arteriosus, fibroelastosis, truncus arteriosus or Ebstein's anomaly In contrast to this, anomalous pulmonary venous connection and pulmonary atresia were seen more often in association with PLSVC than a mere chance relationship would have provided

Analysis of the frontal plane P-wave axis was possible in 37 patients In 12 patients no ECGs were available mainly because these patients had died immediately on birth A total of 22 patients had an intermediate P-wave axis suggesting sinus rhythm Two patients had right axis deviation of the frontal plane P-wave and 13 had left axis deviation compatible with a diagnosis of coronary sinus rhythm The ECGs used for analysis had all regular rhythms and normal P-R intervals

DISCUSSION

PLSVC is usually a harmless phenomenon, but can be of importance to the patient Usually is it without haemodynamic significance, but draining into the left atrium may cause central cyanosis Its presence can make cardiac catheterization impossible or at least very difficult from the left arm, and attempts to overcome an obstacle at the level of the anastomosis between the left subclavian vein and the jugular vein have led to a fatal outcome in at least 2 patients reported in the lite-

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PLSVC is one of the most frequent anomalies of the venous system (6). Keith et al. (11) found this anomaly in one per 350 autopsies giving an incidence of 0.3% which is in good agreement with Steinberg et al. (17) who estimated that PLSVC was present in 0.5% of the population. A higher incidence has been found in patients with congenital heart disease. Campbell & Deuchar (2) found this anomaly in 3% of 1500 patients with congenital heart disease and Fraser et al. (5) found an incidence of 4.3% among 703 patients. Our finding of an incidence of 1.3% of PLSVC among 3761 paediatric patients with congenital heart disease is somewhat less than what has previously been reported, but the ages and selection of the patients have not been identical in the various series.

Gensini et al. (6) found a high coincidence of anomalous pulmonary venous connection and atrial septal defect in patients with PLSVC. In only 2 out of 37 patients was PLSVC found as an isolated anomaly. Among 30 patients with PLSVC, Fraser et al. (5) found 9 patients with atrial septal defect, 7 patients with tetralogy of Fallot and 6 patients with anomalous pulmonary venous connection. One patient had no associated congenital heart disease. Momma & Linde (13) had 26 patients with PLSVC. Ten patients had atrial septal defect and 8 patients tetralogy of Fallot. Three patients had anomalous pulmonary venous connection and in one patient there were no associated congenital heart disease. Our results confirm the frequent association between PLSVC and atrial septal defect. Among our 49 patients there were 19 with atrial septal defect which means that this anomaly is found in approximately 40% of patients with PLSVC encountered during cardiac catheterization or at autopsy. However, when patients with isolated atrial septal defect were considered, the association with PLSVC was not greater than expected by a mere chance relationship (Table 1). The high coincidence of atrial septal defect and PLSVC among patients with congenital heart defects is therefore most likely due to the overall high incidence of atrial septal defect. Only in patients with anomalous pulmonary venous connection and patients with pulmonary atresia was a higher association with PLSVC than expected by a mere chance relationship found. The unexpected high incidence of associated defects in the pulmonary veins and in the systemic veins is, however, well understood when one considers the fact that the embryological evolution of the pulmonary venous system takes place simultaneously with the development of the systemic venous return (6). In order not to miss a simultaneous occurrence of anomalous pulmonary venous connection and PLSVC several blood samples should be drawn at various sites between the right atrium and the subclavian vein during cardiac catheterization.

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Our study has confirmed the relatively high incidence of left axis deviation of the frontal plane P wave associated with PLSVC in a paediatric population with congenital heart disease. Among 37 patients with PLSVC associated with other congenital heart defects 13 patients or 35% had this anomaly.

The significance of left axis deviation of the frontal plane P wave in patients with PLSVC remains, however, to be demonstrated Similarly to the finding of a greater amplitude in the left jugular pulse compared with the right (9) and the special radiological appearence of PLSVC (1), a left axis of the frontal plane P wave can act as a clue to the clinical diagnosis of PLSVC What the left axis deviation of the P-wave means in terms of the site for impulse formation and rhythm instability has so far been mostly speculative (7, 13) The finding by Fraser et al (5) of an incidence of supraventricular tachycardia of 38% during cardiac catheterization in patients with PLSVC as compared with one of 8% in other patients submitted to the same procedure suggests, however, electrical instability in the presence of PLSVC And in a boy who suddenly died James et al (10) found a PLSVC as the only macroscopic anomaly Microscopic examination revealed abnormalities in both the sinus node and the A-V node The authors therefore suggested that the function of the sinus node, the A-V node and the His bundle should be evaluated in any patient having PLSVC

ACKNOWLEDGEMENT

We wish to thank the Paediatric departments in the following hospitals Rigshospitalet Kobenhavns Amts Sygehus in Gentofte Odense Sygehus and Aarhus Kommunehospital for access to their patient record files

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Submitted Oct 28 1978
Accepted July 20 1979

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Acta Pædiatr Scand 69. 109–111, 1980

FAMILIAL AGGREGATION OF BLOOD-PRESSURE IN NEWLY BORN INFANTS AND THEIR MOTHERS

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days No significant correlation was found between the SBP in the mothers and the recordings in the infants aged 2–3 days but highly significant correlation was found between the SBP in the infants at 4–5 days and both the SBP ($r=0.54$, $p<0.001$) and the diastolic blood-pressures ($r=0.37$, $p<0.05$) in the mothers Familial aggregation of blood pressure levels has thus been demonstrated in infants at an earlier age than previously reported.

KEY WORDS Blood pressure, familial aggregation, infants

Familial aggregation of blood-pressure in adults has been suggested by clinicians in reports on families in which several members have hypertension (1, 2)

The findings of familial aggregation of blood-pressure have been extended to include siblings as young as two years of age (3) and, recently, significant aggregation has been found in black infants of one month of age (4)

The purpose of this investigation was to show how early it was possible to demonstrate familial aggregation of blood pressure

MATERIAL AND METHODS

A group of 48 women and their newly born infants admitted to the Obstetric Department, Frederiksberg Hospital Copenhagen were registered in a consecutive investigation

In order to eliminate factors which might cause variations attempts have been made to make the material as uniform as possible All of the blood pressures were recorded by the same individual with the same apparatus, at the same time of the day between 10 and 12 a m and between two meals The systolic blood pressures were measured on two occasions in the infants on the 2nd or 3rd day and on the 4th or 5th day after delivery Blood pressures were measured with the Arteriosonde 1020*, using the Doppler technique (5), and a cuff 4.5 cm in width and 9.5 cm in length

Close aggreement was found between systolic blood pressure measurement with this instrument and simultaneously direct intra aortic measurement in infants (6)

The conscious infant was placed on his back with the right arm extended Gelisonde, an ultrasonic coupling medium, was applied to the transducer The cuff was applied firmly round the upper arm above the antecubital space with the transducer over the brachial artery The cuff was then inflated to approximately 20 mmHg above the point at which the hissing sound ceased at a rate of 2–3 mmHg per second Blood pressure levels corresponding to the first Korotkoff sound were recorded The results are the mean of three measurements

In the mothers blood pressures were also measured with the Arteriosonde, using a cuff of 12 cm in width The mothers rested in bed for a period of at least five minutes before blood pressure measurements were recorded

Infants with birthweights of under 2500 g, infants born by caesarean section, infants with infections or feeding problems were excluded from the material Mothers taking medicine for hypertension in the pregnancy were also excluded

RESULTS

The mean blood-pressure readings and the standard deviations in the mothers and the newly born infants are shown in Table I

Table I. *Analysis of correlation of blood pressure in newborns and their mothers*

	No.	Systolic blood pressure Mean	S.D.	Correlation	r	p	
Infants							
2–3 days old	28 boys	67	12.3	Mother systolic/ Child systolic	0.11	Non significant	(♂ Non significant ♀ <0.05)
	21 girls	67	9.8	Mother diastolic/ Child diastolic	0.15	Non significant	(♂ <0.01 ♀ Non significant)
4–5 days old	26 boys	74	10.7	Mother systolic/ Child systolic	0.54	<0.001	(♂ Non significant ♀ <0.01)
	17 girls	71	9.8	Mother diastolic/ Child systolic	0.37	<0.05	(♂ <0.05 ♀ Non significant)
Birthweight	♂ 2 650–4 650 $\bar{x}$=3 270	♀ 2 750–4 550 $\bar{x}$=3 490					
Mothers	No.	Mean	S.D.				
Systolic blood pressure	48	110	13.8				
Diastolic blood pressure		63	11.0				

The systolic blood-pressures of the infants were found to increase in both sexes from the first measurement at 2–3 days old to the second measurement at 4–5 days old. No differences in the blood pressure levels in the two sexes were observed.

An analysis of the correlation (correlation coefficient=r) between the newly born infants and their mothers was undertaken. No significant correlation was found at the age of 2–3 days but highly significant correlation was found at the age of 4–5 days, both as regards the systolic ($r=0.54$, $p<0.001$) and the diastolic ($r=0.37$, $p<0.05$) blood pressure of the mothers. In girls, significant correlation to the systolic blood pressure of the mothers was encountered both at 2–3 days and 4–5 days while boys had significant correlation to the diastolic blood pressure of the mothers at the same ages.

DISCUSSION

In this investigation, familial aggregation of blood pressure was found in infants at a younger age than in previous reports.

The failure to demonstrate familial aggregation in infants aged 2–3 days corresponds approximately to the massive physiological alterations which occur shortly after birth. The authors have demonstrated significant correlation between the blood pressures of the mothers and the systolic blood pressures of the infants as early as 4–5 days of age. No explanation has, however, been found for the observations that good correlation exists between the systolic blood pressures of the girls and their mothers and the systolic blood pressures of the boys and the diastolic blood pressures of the mothers.

Mongeau (7) found in his study of older children nearly as high correlation coefficient between fathers and children (systolic $r=0.24$, diastolic $r=0.21$) as between mothers and children (systolic $r=0.27$, diastolic $r=0.26$).

It is tempting to speculate on the fate of infants of parents with high blood pressures. Are some of these children destined to become clinically hypertensive at the ages of forty or fifty years? Would early administration of mild antihypertensive medication reverse this tendency?

It has been found (8) that familial aggregation observed in childhood was reproducible in a follow up study four years later. A study of Air Force recruits (9) followed-up over a period of 30 years, beginning at the age of 24 years, suggests that blood pressure readings at the age of 36 years are predictive of the pressures at 54 years

It is possible that early blood-pressure recordings in infants may provide indications of the risk of subsequent development of essential hypertension In order to test this, a longitudinal study is necessary but it emphasizes the need for measurement of blood-pressures in children and follow-up control of children with high blood-pressures as compared with the normal range for age and sex

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Submitted Febr 20 1979
Accepted July 15, 1979

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Submitted Febr 20 1979
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Acta Pædiatr Scand 69 113–118, 1980

COARCTATION OF THE AORTA

A Follow up Study after Surgical Treatment in Infancy and Childhood

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ABSTRACT Sørland, S J , Rostad, H , Forfang, K and Åbyholm, G (Departments of Paediatrics, Surgery and Medicine, Rikshospitalet, The National Hospital, Oslo 1, Norway) Coarctation of the aorta A follow up study after surgical treatment in infancy and childhood Acta Paediatr Scand, 69 113, 1980 —Between 1951 and 1973, 138 patients aged 0 to 12 years were operated on for coarctation of the aorta Ten of 18 infants died early There were 3 late deaths, 2 occurred suddenly and 1 after reoperation Three of the 125 late survivors had severe, 19 had slight symptoms, while 103 had no complaints, 3 of whom refused examination The remaining 122 ... between 2 and 24 years (mean 10 9 years) after the operation Two pa
hemipareses (trau
ing mmHg, totally
without recoarcta...
18 % of the late survivors It is suggested that the optimal age for surgical repair of coarctation of the aorta is between 4 and 6 years of age A long term follow up is recommended in all patients

KEY WORDS Congenital heart disease, coarctation of the aorta, hypertension, sudden death, spinal cord injury

Since the first surgical repairs of coarctation of the aorta in children and adults in 1944 and 1945 (3, 6), and in infancy in 1950–52 (2, 11), the operation has become a widely accepted form of treatment However, the optimal age for operation is not established, and the long-term prognosis after surgery is only partly known To get more data pertinent to this problem we made a follow up study of all infants and children who had survived coarctectomy in the National Hospital of Norway

MATERIAL AND METHODS

Up to July 1973 138 infants and children were operated on for coarctation of the aorta (Table 1) The first child was treated in 1951 and the first infant in 1962 Of 18 infants 10 died (18) They had in addition shunt lesions pulmonary hypertension and congestive heart failure Standard technique was resection of the coarctation with primary anastomosis Always some sutures were interrupted to permit growth Three patients were operated with a Dacron tube graft end to-end One had an end to-side bypass graft

The follow up study of all the surviving 125 patients was made from 1975 to 1977 from 2 to 24 years (mean 10 9 year
year age
years resp
patients one to be answered by the patient or the parents and the other to be filled in by the family doctor after clinical examination

All patients with cardiovascular symptoms or signs were referred to a paediatricians to specialists in internal medicine or to cardiologists (often to one of the authors) Additional data was given from other hospitals concerned Information about course and symptoms was obtained from all patients Three of them who felt quite well refused examination

The blood pressures (BPs) were measured in supine position after 5 min rest The width of the inflatable cuffs used were about 2/3 of the limb length, and the cuff length at least 2/3 of the limb circumference The diastolic value was recorded at the disappearance of the sounds or if this was not possible, at the muffling of the sounds (13) In some cases the Doppler technique was used

Observed BPs were compared with the data reported by Londe (13) on BPs in normal children up to the age of 15 years, as similar data on a large number of normal Norwegian children still are lacking

Hypertension was defined as BP values exceeding 1) normal average BP plus 2 standard deviations (13), for

Table 1. *Age distribution. Early and late deaths*

	Age at operation (y.)				Total no
	<1	1–3	4–6	7–12	
No. of pats	18	10	27	83	138
Early deaths*	10	–	–	–	10
Late deaths	–	1	1	1	3
Alive	8	9	26	82	125

* Within 30 days after operation

ages up to 15 years or 2) systolic 140 mmHg and/or diastolic 90 mmHg, above the age of 15 years.

Recoarctation was diagnosed when systolic BP in the ... by more than 10 ... patients had car-

RESULTS

History and symptoms

Three patients died during the follow-up period.

Case 1 had a satisfactory coarctectomy at 13 months of age. However, she got a restenosis a year later. At 14 years of age her pressure gradient was 40 mmHg at rest and 108 mmHg during exercise. She was reoperated with a

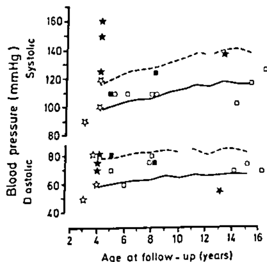


Fig. 1. Systolic and diastolic blood pressure in the right arm at follow up in patients with coarctectomy in infancy (*asterisks*) and at the age of 1 to 3 years (*squares*). Filled symbols indicate patients with recoarctation. Continuous lines indicate average pressures in normal children (13). Broken lines indicate average plus 2 standard deviations.

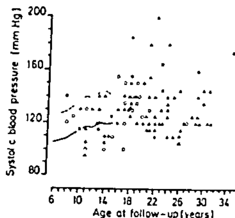


Fig. 2. Systolic blood pressure in the right arm at follow up in patients with coarctectomy at the age of 4 to 6 years (*circles*), and 7 to 12 years (*triangles*). Filled symbols recoarctation. Lines as in fig. 1.

Dacron tube graft from the left subclavian artery to the descending aorta. After 4 weeks she died of uncontrollable bleeding from a branch of the pulmonary artery eroded by the graft.

Cases 2 and 3 were boys, aged 6 and 9 years at coarctectomy. Femoral pulses and arm BPs were normal after 10 months and 6 years respectively. They had no symptoms. Both died suddenly at the ages of 17 and 27 years. Autopsy revealed only slight constriction of the aorta. Case 2 had a markedly enlarged heart with hypertrophy, particularly of the left ventricle. In case 3 an aortic aneurysm had ruptured into the pericardium.

Of the 125 survivors 103 (82.4%) had no complaints. Nineteen patients (15.2%) had slight symptoms (only during heavy exercise) and 3 (2.4%) were severely disabled. Dyspnea occurred in 17 patients, leg pain or fatigue in 16, palpitations in 10 and headache also in 10 patients. Seven and 6 patients, respectively, had dizziness and chest pain.

Cardiovascular findings

Data from physical examination were obtained in 122 of the 125 late survivors. In Figs. 1, 2 and 3, upper limb BP at the last follow-up has been compared with normal values (13).

Twenty patients had systolic hypertension only, in 9 diastolic pressure only was elevated,

Table 2 *Recoarctation and hypertension at follow-up*

Figures within parentheses denote number of hypertensive patients with recoarctation

Age (y) at coarctectomy	<1 (n=7)	1–3 (n=9)	4–6 (n=26)	7–12 (n=80)	Total (n=122)
Recoarctation	4	2	4	12	22
Hypertension	5 (3)	0	8 (3)	22 (8)	35 (14)

and 6 had both systolic and diastolic hypertension Thus a total of 28 6% of the patients were hypertensive

Recoarctation was diagnosed in 14 of the hypertensive and in 8 of the normotensive patients (Table 2) A further 2 patients initially corrected in their second year of life had already been operated on for recoarctation during the follow-up period, with one death The other had normal BP at the last examination. In all, recoarctation thus occurred in 24 (18 7%) of the 128 patients The incidence was 57% among the "infants" and 40% in the "1–3 years" age group In the "pre-school" and 'school age" groups it was the same 15%. In 20% of the patients with recoarctation a *residual* coarctation had been diagnosed early postoperatively

Fig 4 shows that 4 of the 6 patients with a gradient of 35 mmHg or more were operated on in infancy Four patients with a low gradient (up to 20 mmHg) had systolic BP in the arm of 160 mmHg or more They were be ween 7 and 12 years at the time of operation, and their hypertension seems to be more marked than would be expected from the slight gradient.

Hypertension without recoarctation (nor aortic incompetence) was found in 12.5% of those operated on under 3 years of age, and in 17.0% in the older age groups This difference is not significant In the whole material the incidence of pure systolic and pure diastolic hypertension was equal 7 4%. Elevation of both pressures occurred in 2 5%.

Associated anomalies Ventricular septal defect (VSD) and aortic valve lesions were the most frequent (Table 3) Additional cardiovascular surgery was performed during the follow up period in 7 patients: closure of VSD in 2, aortic valvulotomy in 1 and mitral valve replacement in 1.

Neurological sequelae were present in 4 patients. One patient had *paraparesis* at 3 months of age after a complicated repair of coarctation and a long preductal aortic hypoplasia, and pulmonary artery banding because of VSD Later, he had closure of the VSD,

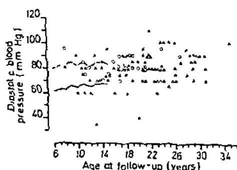


Fig 3 Diastolic blood pressure in the right arm at follow-up after coarctectomy at the age of 4 to 6 years and 7 to 12 years Symbols as in Fig 2

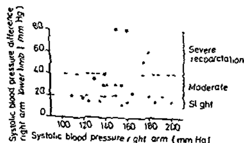


Fig 4 Distribution of arm/leg systolic pressure gradients versus arm systolic pressures and age at operation Symbols as in Figs 1–2

Table 3 *Additional heart lesions at follow-up*

Lesions	No of patients
Mitral insufficiency	4
plus aortic stenosis	1
Aortic stenosis	3
Aortic insufficiency	4
plus ventricular septal defect	1
Ventricular septal defect	8
Atrial septal defect	1

debanding and orthopedic surgery because of contractures

One patient with congenital ptosis had a ductus arteriosus ligated at the age of 4 months. He started walking normally After coarctectomy and ligation of 3 collaterals at 14 months of age he walked with extremely *outwardly rotated legs*

Two patients operated on at school age had *hemiparesis* not related to the coarctectomy In one it was traumatic The other had a late cerebrovascular accident, with mild to moderate recoarctation and probably a stenotic carotid artery

DISCUSSION

In the evaluation of the long-term outlook after surgical repair of coarctation of the aorta, a retrieval of *all* the patients is of great importance This has not been achieved in previous reports (12, 14, 17, 21 22) In the present report the fate is known of *all* the 128 patients surviving coarctectomy although 3 patients without symptoms refused examination

Late deaths occurred in 2 3% of our material Other reports show late deaths rates between 5 1 and 8 4% after coarctectomy in infancy and childhood and between 13 and 16% after operation in adolescense and adult hood (5, 14, 17, 21, 22) These late deaths are often associated with known coexistent heart lesions or recoarctation Some deaths are sudden and unexpected (22) and may be caused by rupture of an aortic aneurysm (17), as in one of our patients

Recoarctation was diagnosed in accordance with most recent authors (14, 16, 21) a systolic pressure arm/leg differential exceeding 10 mmHg, or usual clinical criteria After coarctectomy *between the ages 1 and 12 years* the total rate of recoarctation amounted to 17% in our series, compared to rates in other reports (16, 20, 21) of between 5 and 12 6%

Using a pressure gradient exceeding 20 mmHg (12, 22), the incidence of recoarctation in our material was 16% in the age group 1-6 years and 3.7% among the older ones, compared to 9 and 5%, respectively, in other series (12)

After coarctectomy in *infancy* our recoarctation rate was high 4 out of 7 patients (57%), compared to 16 and 40% in larger series (4, 12, 17) The difference may be explained by our limited infant experience and that the use of patches was started later

Recoarctation may actually be *residual* coarctation, or true *restenosis*, which in infants may occur already within 48 hours after a primarily satisfactory anastomosis (17, 22) More often restenosis develops gradually over months or years (8, 22) The longer observation time in our series compared to previous ones may contribute to our higher rate of recoarctation In our series the shortest follow-up time was 2 years, with a mean nearly 11 years Other reports (4, 16, 20) include patients followed for only 6 months with mean follow up time between 2 and 5½ years

Operation for re coarctation is recommended in patients with arm/leg pressure gradient of at least 35–40 mmHg (4, 8) According to this 6 of our cases may need reoperation in addition to the 2 who already have been reoperated on

Systolic and diastolic hypertension without re-coarctation was in our material distributed in accordance with Nanton & Olley (16) Other authors (17, 20) have reported an increased incidence of systolic hypertension with age at coarctectomy

Blood pressure studies during exercise (performed in only a few of our patients) may often

reveal a significant pressure gradient not present at rest (9) Some patients without gradients or aortic incompetence may have inappropriate elevation in systolic pressure with mostly unchanged diastolic pressure, suggesting a normal peripheral small arterial resistance, but an altered compliance of the aortic arch and major vessels (10)

Spinal cord injury was found in 2 of our patients (1 6% of the early survivors) One infant aged 3 months had *paraparesis*. Poor collaterals and clamping of aorta twice may have contributed to the injury. The other patient had *adductor paresis* after an uncomplicated coarctectomy at the age of 14 months. In a large collected series of 12 532 cases spinal cord injury was found in 0 41%, the youngest patient being 3 years old (1)

The optimal age for operation of coarctation of the aorta has been debated. In infants with refractory congestive heart failure emergency surgical repair is now unanimously recommended Associated heart lesions may contribute to the indication for early operation When symptoms, hypertension and associated lesions are mild or not present, the optimal time for surgery is stated to be between 7 and 20 years (15, 19), because of the increased mortality and recoarctation rate of earlier operations Our data may indicate an optimal age of 4–6 years for elective surgery, in accordance with Nanton & Olley (16)

Operated patients should have regular follow-up, with special emphasis on hypertension, recoarctation, other aortic disease including valvular lesions and myocardial disease Exercise studies and echocardiography may here be of great value Many patients would probably also need catheter studies to get a basis for management of potential future complications

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Submitted Febr 21, 1979
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LETTER TO THE EDITOR

Sir

We are taking the liberty of writing to question the emphasis given by Dr Hovi and colleagues in their recent paper in *Acta Paediatr Scand* *68* 567 1979

It seemed to us that in the discussion there was some confusion concerning the standards or levels by which they considered the adequacy or otherwise of riboflavin in human milk For example, they used the phrase since the concentration in riboflavin is relatively low in human milk and later on, levels of riboflavin in human milk may be marginal'

To us this seems a back to front way of putting matters In fact the RDA (recommended dietary allowances) for riboflavin or, indeed, for any other nutrient for the young baby are based on *the amount of that nutrient found to be present in human milk* However, in the totally different circumstances of the ingredients in cow s milk based formulas the level of many nutrients is increased to give a level of safety This seems to us to be a recurring error in thinking which we have referred to as ' The Great RDA Hoax" (*1*) that is that RDA are based on human milk, but human milk is deficient For example, the authors say ' further information is also needed to determine whether riboflavin supplementation might be beneficial in healthy human milk fed newborns ' is really a long way from being biologically based

At the same time, the authors' studies on the possible effects of phototherapy on different aspects of body metabolism, including riboflavin are indeed most timely

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Acta Pædiatr Scand 69 121–122, 1980

SHORT COMMUNICATION

UNRESPONSIVE NEONATAL ASPHYXIA ASSOCIATED WITH CONGENITAL PLEURAL EFFUSION

In newborn infants, transient tachypnea and β streptococcal pneumonia are frequently accompanied by small collections of pleural fluid Larger amounts are found in severe erythroblastosis fetalis However, congenital chylothorax is the most frequent cause of clinically significant pleural effusion in the newborn (2) From April 1976 to October 1978, we recognized and treated four cases of spontaneous bilateral chylothorax In all infants the pleural effusion was present at the time of birth and resulted in a profound neonatal asphyxia All four cases were strikingly similar and their clinical data are summarized in Table 1

Comments

Neonatal asphyxia is usually a consequence of fetal distress of various etiologies or the result of pharmacologic depression of the infant s respiratory center due to the administration of drugs or anesthesia to the mother Among the cases of severe neonatal asphyxia (Apgar score ≤3 at one minute or ≤6 at 5 minutes), 4 patients who were subsequently diagnosed as having bilateral pleural effusion had unexpected low Apgar scores No important prenatal risk factors were recognized in any of the mothers Two cases were monitored with external fetal monitors, and one with an internal monitor In none of these cases was fetal distress demonstrated None of the mothers received any drugs known to cause respiratory depression In every case the resuscitation included assisted ventilation, volume expansion, and correction of the metabolic acidosis, yet failed to improve the infant's respiratory condition Because of persistent hypoxemia, hypercarbia, and severe repiratory distress, chest X-rays were ob-

Table 1 *Clinical findings in four infants with congenital pleural effusion*

	Case no 1	Case no 2	Case no 3	Case no 4
Birth weight (g)	2460	2540	2990	3160
Gestational age (wks)	34	36	38	37
Sex	Male	Male	Female	Male
Type of delivery	Spontaneous vaginal	Spontaneous vaginal	Spontaneous vaginal	Spontaneous vaginal
Apgar score (1 and 5 minutes)	5–2	3–3	0–3	1–1
Volume drained with initial thoracocentesis	80 cc	90 cc	215 cc	180 cc
Decrease in oxygen requirement following initial thoracocentesis	From 1 0 to 0 72	From 1 0 to 0 03	From 1 0 to 0 35	From 1 0 to 0 38
Follow up	Normal at 16 months	Normal at 12 months	Normal at 15 months	Mild seizure disorder Normal at 9 months

tained and showed, in each instance, bilateral pleural effusions Large collections of yellow ish fluid were initially drained in each case by thoracocentesis or continuous chest tube aspiration In each case the lack of effective spontaneous respiration and the failure to respond to the resuscitation were caused by the copious collection of pleural fluid com pressing the infant s lungs This hypothesis was supported by the immediate clinical im provement and the decrease in the require ment of oxygen and assisted ventilation which followed the drainage of the fluid by thoraco centesis or continuous chest tube aspiration in each patient (see Table 1)

The fluid cell profile showed ≥90% lympho cytes, confirming the diagnosis of chylothorax in the 4 infants The differential diagnosis of this and other types of pleural effusion we have discussed previously (6) and was also reported by Brodman in his review of 34 cases (1) The subsequent management of neonatal chylothorax has also been extensively dis cussed (1 3–6)

Our 4 patients survived One infant subse quently developed a mild seizure disorder Three were considered normal at follow up evaluation at ages 9 to 16 months The aware ness of pleural effusions as one of the causes of nonresponsive neonatal depression fol lowed by the radiological demonstration and the immediate treatment with thoracocentesis or the placement of chest tubes is essential to save the lives of these critically ill newborn infants

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CASE REPORT

DEFICIENT CELL IMMUNITY AND MILD INTERMITTENT HYPERAMINOACIDEMIA IN A PATIENT WITH THE RUBINSTEIN TAYBI SYNDROME

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From the Divisions of Genetics and Hematology and Experimental Pathology Subjefatura de Investigacion Cientifica Unidad de Investigacion Biomedica Centro Medico de Occidente Instituto Mexicano del Seguro Social Guadalajara Jalisco Mexico

ABSTRACT Rivas F, Fragoso R Ramos-Zepeda, R Vaca, G, Hernandez, A,

Guadalajara, Jalisco, Mexico) Deficient cell immunity and mild intermittent hyperaminoacidemia in a patient with the Rubinstein Taybi syndrome Acta Paediatr Scand,

heterogeneity of RTS

KEY WORDS Rubinstein Taybi syndrome deficient cell immunity, hyperaminoacidemia

Since the first description by Rubinstein & Taybi (7) of a syndrome characterized by mental retardation short stature peculiar facies, and broad thumbs and big toes more than 200 cases have been reported (3) The purpose of this communication is to describe a patient with the Rubinstein Taybi syndrome (RTS) in whom deficient cell immunity and intermittent hyperaminoacidemia were ascertained

CASE REPORT

A Mexican boy aged 2 years 8 months (Fig 1) was the product of a full term uncomplicated fourth pregnancy and normal delivery Birthweight was 4020 g length was not recorded Recurrent respiratory and gastrointestinal infections feeding difficulties and delayed psychomotor development (DQ 35) were observed since early infancy

Immunizations He received TOPV and DPT (three doses of each during the first 6 months of life) Smallpox and measles vaccines were administered at about one year of age There was no reaction following the smallpox vaccination and there is no observable scar

Physical examination revealed height of 79 cm weight of 9 5 kg and cephalic circumference of 44 cm (all values below the third percentile) Clinical and radiological findings are summarized in Table 1 The thorax roentgenograms showed images compatible with calcified pulmonary tuberculosis The EEG was suggestive of mild non specific cortical and subcortical diffuse damage No other associated pathological findings were observed

Laboratory data Hemoglobin concentration was 90 g/l with an hematocrit of 32 Urinalysis serum glucose urea and creatinine X-chromatin and karyotype (G bands) were normal for his age and sex Screening tests for inborn metabolic errors were positive for tyrosine or its derivates (Millon test) and for homocystine (Ag nitroprusside test) in two out of three different random samples ferric chloride and phenistix tests were negative p-OH phenyl lactic and p-OH phenyl acetic acids were identified in urine by means of paper chromatography Three plasmatic aminograms from samples taken on different days revealed the following levels tyrosine 155 61 and 116 μmol/l (normal range for his age 33–75 μmol/l) phenylalanine 145 54 and 157 (normal range 45–65) and threonine serine (no distinguishable peaks) 419 161 and 615 (normal range 157–374) the remaining amino acids were within normal limits Beta hemolytic streptococci were identified in a culture of pharyngeal secretions and a level of 333 Todd units of antistreptolysins was found

tained and showed, in each instance, bilateral pleural effusions Large collections of yellowish fluid were initially drained in each case by thoracocentesis or continuous chest tube aspiration In each case the lack of effective spontaneous respiration and the failure to respond to the resuscitation were caused by the copious collection of pleural fluid compressing the infant's lungs This hypothesis was supported by the immediate clinical improvement and the decrease in the requirement of oxygen and assisted ventilation which followed the drainage of the fluid by thoracocentesis or continuous chest tube aspiration in each patient (see Table 1)

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Table 1 *Clinical and radiological findings in the patient*

Clinical	Radiological
Flat occiput	Microcephaly
Prominent forehead	Bilateral parietal foramina
Arched eyebrows	Delayed ossification of sutures
Long eyelashes	Wormian bones
Antimongoloid slant of palpebral fissures	Normal foramen magnum
Bilateral epicanthi	Thin ribs
Ocular hypertelorism	*Spina bifida at L 5 and S-1*
Convergent left strabismus	Narrow pelvis
Parrot like nose	Thin and osteoporotic long bones
Broad nasal bridge	Enlargement of last phalanges of fingers and toes principally of thumbs and great toes
Nasal septum below alae nasi	Moderately retarded bone age (2 years)
Long and wide philtrum	
Small mouth with thin upper lip	
Highly arched palate	
Short neck	
Pectus carinatum	
Bilateral cryptorchidism	
Saddle like and hypoplastic scrotum	
Pes planus	
Generalized hypotonia	
Hyperextensible joints	

tests were performed The low phagocytic index the unresponsiveness to delayed hypersensitivity tests, mainly to PPD and streptokinase streptodornase (considering the probable primary pulmonary tuberculosis the levels of antistreptolysins and the demonstration of beta hemolytic streptococci from the child s pharynx) and the low percentage of rosette forming lymphocytes strongly suggest deficient immunity principally of the cellular type a hitherto undescribed feature in RTS Nevertheless the deficiency should be partial, since the DNFB test was positive

The positive tests for tyrosine derivates and homocystine in urine as well as moderately high plasma levels of phenylalanine threonine and serine in two out of three occasions have not been previously reported in RTS although nonspecific aminoaciduria (6) and glycinuria (3) have been found The meaning of these intermittent probably non specific hyperaminoacidemia and aminoaciduria can not be fully explained with the present information Although a coincidental factor can not be completely ruled out, the finding of deficient cell immunity and mild intermittent hyperaminoacidemia and aminoaciduria can constitute further evidence of the phenotypic, and probably also genetic (2), heterogeneity of RTS The first feature could explain the frequent infections of patients with RTS and should be kept in mind when studying and treating cases with such a syndrome Nevertheless, further studies to delineate the immunological status of patients with RTS are necessary

ACKNOWLEDGEMENTS

This work was supported in part by a grant from the Ford Foundation We thank Zamira Nazará for the roentgenological interpretation Hector Gomez Estrada and Amado Gonzalez Mendoza for critically reviewing the manuscript and Margarita García Aceves for secretarial assistance

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Submitted March 22 1979
Accepted May 25 1979

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Fig 1 The patient Photographic composition of face hands genitalia and feet

tion of immunoglobulins by single radial immunodiffusion showed IgG 11 8 IgA 0 46 and IgM 1 6 g/l Anti A and Anti B blood group (the patient was O) isoagglutinines titers were 1/8 and 1/16 respectively A chemotaxis test (9) yielded normal results Polymorphonuclear phago cytosis was evaluated by ingestion of latex particles (9) and the average number of granules incorporated into the cells was 8 4 (normal 22+4) The nitroblue tetrazolium reduction test (5) and the T lymphocyte transformation after stimulation with phytohemagglutinin (4) were nor mal The percentage of rosette forming lymphocytes (1) was 12 (normal range 36–70) Delayed hypersensitivity was tested on two different days one month apart with PPD (2 IU and 4 IU) candidin streptokinase strepto dornase histoplasmin and coccidioidin Intradermo reactions were negative after 24 48 and 72 hours A sensitization test was performed with 0 05 ml of a 30% solution of dinitrofluorobenzene (DNFB) in acetone applied to the volar surface of the left forearm on a piece of filter paper 1 cm in diameter for three days a challenge doses of 0 02% was used on the right forearm A mild erythematous area of 0 6 mm in diameter was seen on the left side while no reaction was observable on the right one One month later the sensitizing agent was reapplied in the same way using concentrations of 0 06 and 0 02% (left and right forearm) for three days after which an erythematous indurated area 1 cm in diameter was note on both sides

Family data The non consanguineous and norm parents were aged 26 (mother) and 24 (father) at the pa tient s birth Three maternal half sibs and a sister we also normal Cultures of pharyngeal secretions from bot parents showed beta hemolytic streptococci The ch ld paternal grandmother suffered active pulmonary tuber culosis when the patient was an infant and she was in con stant contact with him during his first six months of l fe The same immunological studies performed in the propo situs were carried out in both parents and his sister w th results within normality

DISCUSSION

The diagnosis of RTS in the patient was es tablished on the basis of clinical and roent genological data In order to investigate a pos sible underlaying factor which could explain the recurrent respiratory and gastrointestinal infections in our patient and consistently present in the syndrome (8), immunological

ANNOUNCEMENT

ANNOUNCEMENT OF PRIZE COMPETITION FOR 1980 AND 1981

The Central Association of Swiss milk producers CH 3000 Bern 6 announces the competition for the international prize for modern nutrition which it is donating for 1980 as well as 1981 The prize amounts to SFr 15 000 – and will be awarded to a scientist from a member state of the international dairy farming association Member states are Algeria Australia Belgium Brazil Federal Republic of Germany Chile Denmark Finland France Great Britain India Iran Ireland Israel Italy Japan *Canada Kenya Luxemburg Malta New Zealand* The Netherlands Norway Austria Poland Sweden Switzerland Spain South Africa Czechoslovakia Soviet Union

Topic for 1980 Nutrition and brain development Deadline February 15 1980

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Authors of articles dealing with the topics in question are invited to submit the following documents in triplicate curriculum vitae bibliography reprints of the most relevant articles related to the prize topic which have been published during the past 5 years The documents should be written in German French or English and sent to the *President of the jury Prof Dr H Aebi Medizinisch* chemisches Institut der Universitat CH 3000 Bern 9 Switzerland

ERRATUM

In the article Hyperlipoproteinemia in newborn infants published in the September issue vol 68 pp 683–690 1979 the second line of the HDL column in Table 4 should read 0 93 instead of 1 93

To John Lind at his reception of the Rosén von Rosenstein Medal, May 1979

John Lind
a fellow and a character in the midst of us clearsighted, intuitive, full of playful seriousness and kindness In this spirit you have —together with a countless number of collaborators—explored many disparate fields *the circulation of the newborn* his first breath and the phonetic pattern of his cry, play therapy father s care of his newborn, and music for unborns The examples show the wide scope of your interests, all studies carried through with an inspiring leadership which has made you not only to an admired scientist, but also to a loved one No phenomena in infants have for you been so self evident that they have not been worth keen observation from minute calcifications in the newborns vessels to the influence of a Japanese baby carrier on parent–infant interaction In every field you found precious grains

The comprehensive and penetrating studies of the physiology of the human placental fetal, and neonatal circulation are the corner stones of your research You have provided direct and eloquent confirmations of many basic theories on the subject In a famous and dramatic suite of pictures you and Carl Wegelius literally visualized the most critical moment in life *the infant s first breath Your ideas and* observations in this field have stirred new thoughts and inspired research throughout the world

In the sixties you published together with Ole Wasz Hockert a now classic booklet on the phonetic pattern of infant cry, where you with skill and fantasy advanced the idea of the cry as a signal with survival value What an odd idea! *But to have success* one should *appear* somewhat crazy but *be* wise You fulfill *the criteria and now, ten years later,* there is an international society for the study of infant cry, publishing its own journal Indeed, you got many children in your cohabitation with science And are still producing new ones

Play therapy, however, is not *your* child But you made it an eminent topic in distinguished scientific societies The play which heals has helped and comforted children all over the world Your introduced rooming in and educated fathers in the maternity ward in the early sixties A bright idea—15 to 20 years *ahead of its time With poets, dreamers,* and artists you share the ability to perceive the tides in our society and the needs of its members, long before they are apparent to others

At last Rosen von Rosenstein who published many of his thoughts about children in small almanacs for the people, has a late follower in you In his spirit you have—through the channels of our time—contributed to popular enlightment with generosity and success

Where is the unifying principle in all this activity? Let me suggest the heart—but the heart not only as a pump but as much as a *symbol for love, sympathy,* and understanding Natural science and arts are unified in your research

John you have never liked to talk about yesterday, only about tomorrow I know that people are expecting you in New York, San Francisco, and Skellefteå to start new projects and to test new ideas We wish you good luck

Jan Winberg

9–792872

ACKNOWLEDGEMENTS

The Editorial Board of Acta Paediatrica Scandinavica wishes to express its sincere gratitude to the following persons outside the Advisory Board who have acted as referees during the past year The standard of the journal depends to a very large extent on the skill and interest of these reviewers

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Acta Pædiatr Scand 69 131–136, 1980

MUSIC AND THE SMALL HUMAN BEING

JOHN LIND

When we work today in our hospitals and other institutions with all means available to restore sick children to health and to help those who have acquired a handicap to develop as tolerable an existence as possible, and when we regard such measures as a matter of course, it can then seem incomprehensible that this attitude has not always been a generally accepted point of view

And yet it is of course so that love for every individual child and respect for him or her as an individual—apart from his physical and mental endowments—are rather new phenomenona for us It is, quite simply, a luxury which has become possible in a time and a culture which offers good material conditions for the caring of children, and at the same time quite comprehensive research and enlightening information on children

These good conditions have of course arisen more quickly in some places than in others There have always existed those groups in society and rural districts which have trailed behind because of poverty and ignorance Even in the beginning of the 1800 s, a Swedish country clergyman could talk in a letter about the state of things in his remote parish The farmers are waiting now for the pox, because now there are too many children in the parish

Smallpox the curse of the time, would later disappear in Sweden thanks to Rosen von Rosenstein s pioneer contribution of introducing and, on a larger scale, carrying out vaccinations (16)

But even our children of today have many of the drawbacks of our civilization to struggle against—the picture of our time is, as far as the children are concerned, certainly by no means only bright—so that one can venture to imagine that the children of former times in crowded dwellings and large families had *some* advantages which the children in our time in many cases lack In those families with many brothers and sisters the older children had to learn early to take care of the younger The infants were also there in the center of the family, the brothers and sisters carried them, rocked them, and sang for them Without resorting to romanticizing of "the olden days", one can venture to presume that many infants automatically received a more all round stimulation than in more recent times, when we, in our western culture with the best of intentions, discovered that infants should lie alone, 'undisturbed" as they say We believed, of course, that all that infants needed was food and dry diapers, everything at definite times, and, in addition, sleep and tranquillity Furthermore, we were afraid of "spoiling" babies We believed that, if the infants became accustomed to being lifted up when they cried, to being carried, to sitting on a lap, in general to being included, the child's need for attention would increase One would, as a parent, make a rod for one's back, and would then, consequently, not have anything other to do than attend to the child We regarded the infant as a little package, a package which, for its part did not perceive much of the surrounding world Research on infants during the last few years has given us an unfamiliar understanding of the significance of interaction between the infant and its surroundings (7) We now know that, right from the time of birth, the infant has needs for stimulation, and this applies to sight, hearing, smell, balance, and touch We also know that even

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Lecture give at the meeting of the Swedish Pediatric Society commemorating Rosen von Rosenstein May 11 1979

there is also evidence of the fact that the infant reacts to the mother s voice from the second or third day of life

Does the child remember the mother s voice from the fetal period? Does the voice of the child, *when it cries* out, have anything in common with the mother s so that she recognizes it instinctively? Do relics exist of the sort of wordless communication which enable the young reindeer and its mother to stay together in a charging herd without losing each other? We don t know—yet What we do know is that one of the first and above all most continuous sense impressions which the fetus receives in the womb is *rhythm* It is not a question of auditory impression under the first months of fetal life, but rather more that the fetus' whole existence pulsates in synchrony with the mother s heartbeat, vibrations due to the stream of pulse waves through the large branches of blood vessels in the mother s body

This regular throbbing, largely an unchangeable rhythm, becomes the unborn child s constant companion during the entire fetal period and constitutes a very important part of the fetus environment

It thus seems natural to imagine, that sounds which are characteristically suggestive of heart sounds can be recognized by the child after birth and have a calming effect creating a sense of security (17) Exactly what the unborn child hears in there, within the uterus (from the time its hearing has begun to develop somewhere between the fifth and sixth month of pregnancy) we don t know We know only *that* it reacts to sound The fetus' heartbeat has been recorded, along with changes in the beats' frequency due to the influence of, for example, music

If music is played for the pregnant woman, an increase in heart rate in the fetus begins after a couple of minutes This increase can, clearly, nevertheless be due to the fact that the music affects the mother and via her the fetus —it does not show, consequently, that the fetus reacts to sound

But if music is played for the mother, low and by means of headphones, and a different music is played for the fetus directly by means of a sound box placed over the uterus, on the mother s stomach, the fetus then reacts with an increased heart rate within five seconds (5)

The unborn child reacts to both high and low tones, including tones that are so low that they can't be perceived by the adult The fetal movements also become livelier under the influence of music The reaction depends on how loudly the music is played (sound from without is very much muffled), how suddenly the music crescendos, which sound frequencies are used, and the "state ' of the child

The voice which the unborn child hears most often is the mother s We know that it reaches in to the uterus The child learns to know the mother s voice, and that voice, when it speaks or sings, becomes, like her heart sound, a connecting link for the child between the fetal life and the extrauterine life

Should one therefore, perhaps, begin quite simply to talk with the child and sing for it, even before it is born?—Yes, I think so The nine months of pregnancy usually seem long for the parents to be But pregnancy can be used for something other than to wait for the baby One can begin to relay the message ' long before one meets fact to face When the mother talks with and sings for her unborn child she opens a direct channel to the child and a possibility of continued contact through that channel when the child is born (11–13) To sing for the fetus can prevent a feeling of alienation in the mother—a feeling which many new mothers feel toward their children (9) The relation to the child becomes more natural, and the feeling that it is her child comes more easily That the father and siblings sing for the unborn child is also viewed as positive, even if their voices do not reach the unborn child as strongly and directly as the mother's It provides a feeling of solidarity in an atmosphere of music and creates an interaction between parents and child

A change as great as that which occurs at



Fig 1 (*a*) Girl 25 sec p p , (*b*) girl 6 min p p , (*c*) boy 16 min p p Photo Th Bergman

the premature baby has well-developed senses at birth Research is now aiming backwards, towards the fetal stages, and will be able to clarify more and more the fetus' reactions to its environment both inside and outside the womb

Personally I feel that very likely one of the biggest discoveries in the field of pediatrics in my time is the concept that the newborn child is a small human being, with all its senses developed, open, and receptive (10, 20)

On the maternity ward at Karolinska Hospital we did an investigation of 130 fully developed newborns after normal pregnancy and delivery (1) The infants were photographed at different time periods, from the very instant of birth and up until 2 hours of age The interesting thing was that none of these babies exhibited any facial expressions which indicated anguish or pain but rather the reverse, intensive alertness and openness for impressions (Fig 1)

We now know that the infants' vision prefers the human face (2), that its hearing reacts earliest to the human voice, principally the mother's (6), we know that its sense of balance is prepared for the child's being lifted up, and that this change from the lying to the upright position signifies developmental impulses for the infant's brain (8)

Everything in the infant is thus disposed toward interaction, and even the adult person in the infant's surroundings reacts to the child's signals by wanting to bend over it, talk with it, and lift it up

It seems to me that the topic "Music and the small human being" divides itself naturally into three parts music *by* the small human being, music *for* and music *with* him

The first sound that the newborn baby produces is the birth cry and that is of course the most delightful music one can hear, whether you are the new parents or personnel assisting at the delivery The birth cry is unique—it is never heard again (19) Its message is that the child has been born and is beginning to breathe with its own lungs

Characteristically set apart from the birth cry are the hunger cry and pain cry, different from each other and not as delightful to listen to Even these cries are a type of music with characteristic pitch, frequency, and rhythm And the mother can identify the infant s cries (18) Investigations have shown that she does this, and that it is the specific rhythm in the cry that enables her to recognize it (14) And

The baby itself has, naturally, developed small sounds of its own very early—and not, by any means, only screams As early as perhaps three weeks of age the baby responds to the parents' smiling with sounds, and at 2–3 months the baby is so interested in what it hears that it responds to music and even to recordings of its own cooing with arm and leg movements and new cooing sounds

The human being is biologically constructed to be able to listen and to be able to repeat what he hears, and everyone who doesn't have any direct physical defect in either the auditory or speech organs has these abilities

For most people, music is a need But for the adult person who did not have this need satisfied as a child, it is perhaps not natural to consider the need in his or her own children But to withhold from a child the source of pleasure, stimulation, and comfort that music can be—from the very beginning and throughout one s entire life—is, in a sense, comparable with not teaching the child to talk There is one major difference the parent who cannot speak himself cannot teach his child to speak But, that parent who does not have what we usually call a "singing-voice" or is not what we usually consider ' musical ' can, nevertheless, give his child some form of music

A South American researcher, Ruth Fridman (3, 4), followed three children from birth and studied their musical development In two families, where the parents sang as a rule for their children, she found that the children imitated the parents' voices at the same pitch beginning as early as 4 months of age One child imitated a simple melody at 9 months The other echoed the end of the melody after every verse Between 9 and 12 months of age both children lay and hummed little tunes of themselves They were able, in other words, to sing long before they could talk

It thus happens, very soon and completely as a matter of course, that the parents no longer sing *for* the child but *with* it And soon the child is singing for the parents and for anyone who will listen

A child who lives with music is no longer satisfied with imitating the parents and reproducing words and tones which it hears, but rather soon creates its own words and melodies New stories every time or existing compositions

The baby tries out and exercises his power of speech and his voice, and also investigates other possibilities for making sound banging his rattle, thumping his hands on the table-top, or hitting a spoon against a plate. At the same time the child is training his movements, coordinating and refining them

To make sounds is fun, exciting, and pleasant, whether it happens by means of the voice or another instrument, and best of all is to combine sounds and movements A two-year old who goes around and around the room while rhythmically banging two pot-lids together, at the same time using his lungs full-force to perform a text and melody never heard before—that is musical and choreographic creativity It exists in all children One needs only to take advantage of it and put it on the right track, where it can be developed further Though it is perhaps not so simple after all, when you consider the number of children in whom this creativity is stifled Stifled by thoughtlessness and disinterest from the side of the surrounding adult world

Nowadays we bring up our children here in the country in the most exemplary and scientifically developed way Our children are well nourished, healthy, and physically well-developed

But I would like to see a little, but nevertheless important, "vitamin-enriched" addition to the food for growth which we give our children on their journey through life It was clearly worded several hundred years ago by William Shakespeare, in his play "Twelfth Night", and reads

"If music be the food of love, play on "

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the time of birth is not experienced by the human being more than once in a lifetime The changeover of many functions, the tremendous change of environment, the drastically altered sensory perceptions And the child begins to immediately orient itself within its new world It is dependent upon its surroundings—but by no means passively The child has skills and experiences which it takes along for life outside the womb, and which can be immediately put to use

One of the very greatest changes for the child after birth must be the loss of the rhythm which, by means of the mother's heart beat, breathing, voice, and movements, characterized so completely life within the uterus One day the child lives within this rhythm, the next day it is lying alone in its own little bed It lies there in what we call "peace and quiet" And the child certainly needs rest and "undisturbedness"—also But it needs in addition a gentle transition to the new life, so that some of the good elements of the fetal life can remain Warmth, the well known sounds, the rhythmic movements—the newborn child needs to continue to receive all of this

Earlier generations recognized the significance of rhythm, and infants were put in cradles, rocked, and songs were sung for them in order to calm and comfort them Both these techniques have been out of use but ought to come into favor again

When a baby is born its brain is equipped so that it will be able to learn to speak But the human being has actually one other language, music Of all the different forms of art, music is the most privileged It bridges the gaps between different languages, and can even be experienced by the unborn and newborn child One need not wait until the baby has achieved a particular level of maturity in order for it to experience music

When one sings or hums, one expresses more—and more direct—feelings than when one speaks When one talks and crows to a little baby, one unconsciously adjusts the voice in a special, melodic, half singing way Without being conscious of it, we choose a channel of communication which leads directly to the child And the infant has a highly developed sensitivity for feelings, for tensions in the air, as well as for harmonic atmospheres There is no better way to evoke a relaxed atmosphere than to sing for the child And one should sing oneself Radios and record players certainly offer sound experience and rhythm, but they lack that which is most important for the child at this stage the warm, direct contact Or, as a somewhat older child expressed it 'I can't sit on the tape recorder's lap "

When one sings, it is all the more positive if one chooses to sing the songs that one sang before the child s birth And that forms of music other than the parents' songs can give lasting memories from one life to another is clearly shown in the following two stories

One mother wrote to me and related During my first pregnancy I received as a present a recording of a Bach suite I played it at least once a day, sometimes more often It became very precious to me, but after my son s birth I put it away among cherished keepsakes

When my son was 7 years old he happened to find the record and played it He then came rushing up to me exclaiming that he had found a wonderful record We played it and listened to it together, and there was no mistaking his excitement, enthusiasm, and rapture Now the boy is grown, and he has never stopped loving that record, which he recognized' once

A conductor, leader of an orchestra in Canada and England, relates that he went back time and time again to a special piece of music without really being able to understand why On one occasion he talked about this with his mother—also a practicing musician—and she then remarked that the matter was easily explained it was the piece of music that she had played most often during her pregnancy before his birth (15)

If one observes an infant while one is singing for it, it is evident that the child exhibits interest for the voice from the very beginning

The baby itself has, naturally, developed small sounds of its own very early—and not, by any means, only screams As early as perhaps three weeks of age the baby responds to the parents' smiling with sounds, and at 2-3 months the baby is so interested in what it hears that it responds to music and even to recordings of its own cooing with arm and leg movements and new cooing sounds

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Acta Pædiatr Scand 69, 137–142, 1980

CHILDHOOD HYPERTHYROIDISM

Results of Treatment

JORMA MAENPAÁ and ANU KUUSI

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ABSTRACT. *Maenpaa, J. and Kuusi, A.* (Children's Hospital, University of Helsinki and Aurora Hospital Helsinki, Finland) Childhood hyperthyroidism. Results of treatment. Acta Paediatr Scand, 69 137, 1980.—40 hyperthyroid children were followed for 0.2–13 (mean 4 5) years The treatment was antithyroid drugs in 20, subtotal thyroidectomy after a drug trial in 18 and primary thyroidectomy in 2 patients. 4 patients who relapsed (3 after surgery and 1 after a drug trial) were given radioiodide 11 of the surgically treated glands were nodular. At the follow-up study 24 patients were euthyroid, 7 were on thyroxine therapy and in 5 others hypothyroidism was discovered. 2 subjects were still on antithyroid drugs and 2 relapsed In 5 euthyroid patients the TRH test revealed a low thyroid reserve. In 28 of 34 subjects examined circulating antibodies to thyroid microsomes were present in high titres. Evidently, regular follow-up is needed because of the high risk of hypothyroidism.

KEY WORDS Hyperthyroidism, Graves' disease, hypothyroidism, thyroid antibodies, children

Childhod hyperthyroidism is usually due to Graves' disease Its treatment is controversial In an earlier series we showed that thyroidectomy was required in 54% of our patients (13) We believed that this was due to thyroid nodules found in over half of the patients Since that time the iodine intake of the population in Finland has increased through consumption of iodized salt, and endemic goitre has greatly diminished (11) Autonomous hyperfunctioning thyroid adenoma is rare, but still does occur even in childhood (20, 21)

This is a report of our experiences and the results of the treatment of childhood hyperthyroidism

PATIENTS AND METHODS

Of 40 patients (Table 1) treated for hyperthyroidism at these hospitals in 1965–1976, 37 have now been examined by us Information concerning the remaining three patients was obtained from the patients themselves and from case records The clinical diagnosis (1, 18) had been confirmed by at least one of the following measurements of serum concentrations of protein bound iodine, butanol extractable iodine, total thyroxine (T_4), free thyroxine (FT_4), and total triiodothyronine (T_3), T_3 Sephadex uptake test, T_4 Sephadex uptake test, plasma thyrotrophin (TSH) response (ΔTSH) to thyrotrophin releasing hormone (TRH), the radioiodine uptake test, and the T_3 suppression test (14) Circulating thyroglobulin and microsomal antibodies were assayed at the Department of Serology and Bacteriology, University of Helsinki, by the passive haemagglutination technique (PH) (15, 16) and microsomal antibodies also by the complement fixation test (CF) (16)

Table 1 *Age and sex of 40 hyperthyroid children and adolescents at the time of diagnosis*

Age, years	No of patients Female	Male	Total
3 41– 4 99	2		2
5 00– 9 99	11	2	13
10 00–14 99	20	2	22
15 00–15 95	2	1	3
Total	35	5	40

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le 4 *TSH response to TRH in 24 children and adolescents after treatment for hyper-oidism*

	TSH reponse			
e of therapy	Subnormal (ΔTSH <3 mU/l)	Normal (ΔTSH 3 0–30 0 mU/l)	Exaggerated (ΔTSH >30 0 mU/l)	Total number of patients
thyroid drugs	2	8	3	13
roidectomy	2	2	7	11
	4	10	10	24

m a very remote place and primitive social nditions

Complications of surgery Apart from hypo yroidism the only complication was a uni-eral transient laryngeal nerve paralysis in e patient

esults of therapy

roup I (Table 2) One patient had a relapse aring the follow up study In three patients ie results of all thyroid function tests, in luding the basal serum-TSH, were normal, xcept for the ΔTSH, which was exaggerated 31 4–89 2 mU/l) (Table 4) In two clinically euthyroid patients with normal results of hyroid function tests the ΔTSH was sub-normal Soon after the follow-up examination one of them became clinically hyperthyroid

Group II a (Table 2) Four of the surgically treated patients and three of those treated with surgery and radioiodine, were on thyroxine therapy because of permanent hypothyroid-ism During this study hypothyroidism was verified in a further five patients They had subnormal serum-T_4 levels and an exaggerated TSH response to TRH stimulation (basal serum TSH level 16 0–138 6 mU/l, normal 1 6–6 9 and ΔTSH 31 5–141 1 mU/l (Table 4) Two patients with normal results of other function tests had an exaggerated response to the TRH test, demonstrating a low thyroid reserve One subject was euthyroid, but had a subnormal response to TRH

Group II b (Table 2) One of the subjects was euthyroid 12 years after surgery The other, a young lady whose toxic adenoma had been enucleated 10 years earlier, had mild thyrotoxic symptoms and eye signs referable to Graves' disease Her serum-T_3 level was 3 47 nmol/l (normal 1 5–2 90 nmol/l), serum-FT_4 concentration 69 pmol/l (normal 36–107 pmol/l) and she showed no response to TRH

Thyroid gland

At the time of diagnosis the thyroid glands were enlarged in all cases The estimated weight of the gland was below 40 g in 23 in-stances and above that in the remaining 17 At the time of follow-up the weight of the thyroid was estimated to be 30 g in four and below that in the others The thyroid was nodular in 11 cases In three of them nodules were found by palpation Histological ex amination of the 20 operated glands revealed nodular goitre in nine, differentiated follicular adenoma in one, papillary carcinoma and lymphocytic thyroiditis in one and diffuse hyperplasia of epithelial cells in eight of the glands In addition lymphocytic thyroiditis was found in three and focal thyroiditis in one of the glands In one case no biopsy was available

Thyroid antibodies

Thyroglobulin antibodies in titres of ≥1 25 were detected at some time during the course of the disease in 28 out of 38 patients, and microsomal antibodies (CF) in titres of ≥1 8 in 10 of 27 patients (Fig 1) At the time of

Table 2 *Results of treatment in 40 children and adolescents with hyperthyroidism*

	Group I Antithyroid drug therapy	Group II*a* Antithyroid drug and thyroidectomy	Group II*b* Primary thyroidectomy
No of patients	20	18	2
Euthyroid	17[a]	6	1
Hypothyroid	0	12[b]	0
Relapses	2[a]	3[b]	1
Still on therapy	2	0	0
Duration of follow up, years	0 2–9 2	1 3–6 8	10 2–12 0
(Mean)	(4 1)	(4 4)	(11 1)

[a] 1 patient relapsed was treated with 131 I and is now euthyroid
[b] These relapsed patients were treated with 131 I and are also included in the hypothyroid group

RESULTS

Mode of therapy

Thirty-eight of the 40 patients were given a trial with antithyroid drugs, two patients were operated on primarily For various reasons 18 patients were later submitted to subtotal thyroidectomy

Medical treatment Twenty patients (Group I) were treated with antithyroid drugs, two of them are still on therapy (Table 2) Four had two courses of treatment and one of them, a mentally retarded microcephalic boy, had radioiodine therapy Carbimazole was used in 2–4 daily doses, initially 20–40 mg/d The dosage depended on the severity of the illness and the size of the goitre In some cases potassium iodide was administered during the first six weeks of treatment In recent years propranolol has been used to alleviate thyrotoxic symptoms

When stable euthyroidism had been achieved, l thyroxine was added in doses of 50–200 μg/d Six months after the start of the therapy, the carbimazole dose was cut down to the maintenance level of 15–20 mg a day The duration of the drug treatment course was 2 98±0 27 years (mean ± S E , range 1 61–6 00 years) The treatment was stopped gradually by reducing the dose of the antithyroid drug The therapy was not discontinued during a major physical or psychosocial stress such as the growth spurt or a change of school

Side effects of antithyroid drugs Carbimazole was generally well tolerated Gran cytopenia occurred in one patient and f and urticaria in another These patients v placed on propylthiouracil In the child urticaria this caused jaundice

Surgical treatment Eighteen pati (Group II*a*) were operated on after one patients), two (three patients) or three cou (two patients) of antithyroid therapy (Table One of the last-mentioned patients also nee two doses and two others one dose of ra iodine-131 One patient was operated twice The duration of drug treatment cour was 2 90±0 35 years (mean ± S E , ra 0 40–5 48 years) The reasons for surgery presented in Table 3

In two cases (Group II*b*) the primary tre ment was surgery One patient had aut omous toxic adenoma and the other ca

Table 3 *Indications for thyroidectomy in of 40 hyperthyroid children and adolescents*

Indication	No of patie	
Primary thyroidectomy	2	
Autonomous toxic adenoma		1
Social indication		1
Thyroidectomy after antithyroid Drug therapy	18	
Relapses		12
Allergic reactions		2
Granulocytopenia		1
Poor compliance		2
Large goitre		1

lar goitres is a further indication of a radical ierapeutic approach in nodular cases This ombination presents only 1 0% of the sur ically removed thyroid glands in Graves dis ase (2)

The incidence of permanent hypothyroidism vas 32% of all patients followed and 60% f the operated cases This is much more than

15) The increased ...

sm is difficult to explain Possible reasons are nore radical surgery increased incidence of autoimmune thyroiditis and a better diagnostic procedure in the later series

The occurrence of high titres of microsomal antibodies in our patients indicates the pres ence of autoimmune thyroiditis (5) Histo logical examination revealed lymphocytic thy roiditis in four of the 19 resected glands Other authors have found an increased inci dence of postoperative hypothyroidism with histological and/or serological evidence of autoimmune thyroiditis (8 17) and Irvine et al have discovered that hypothyroidism is asso ciated with an increased frequency of thyroid microsomal antibodies (9) Barnes & Blizzard on the contrary suggest a higher correlation with thyroglobulin antibodies than with micro-somal antibodies (1) In our surgically treated patients high titres of thyroid microsomal anti bodies were found in sera of both euthyroid and hypothyroid patients and there was no difference in their titres of antithyroglobulin antibodies

Animal experiments (3) and histopatho-logical studies in man (19) suggest that in creased intake of iodine by the population would result in an increased incidence of auto-immune thyroiditis A striking difference has been found in the incidence of juvenile chron ic *thyroiditis between a seaside* area (5 3 per 1 000) and an urban area (1 4 per 1 000) (7) the authors suppose that one explanation would be a difference in the amount of iodine intake in the form of seaweeds

Hypothyroidism is to be regarded rather as the ultimate result of the disease than as a complication of the surgical therapy Sponta neous hypothyroidism did not occur in the medically treated patients, but the low thyroid reserve shown *by the TRH* test in three pa tients points to this possibility, which was seen in 6% of the adult patients followed by Irvine et al (9) In two patients, followed by Lam berg and *colleagues (12), the response to TRH stimulation* has remained exaggerated for many years although the patients are still euthyroid

Regular follow up throughout life is impor tant, since the diminished thyroid reserve and *active thyroiditis process*, indicated by the presence of thyroid antibodies in high titres, may lead later on to hypothyroidism

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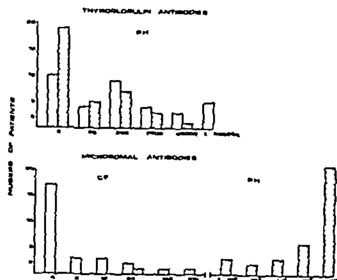


Fig 1 Highest thyroid antibody titres of 38 patients during the treatment (□) and at the follow up (▨)

this follow-up study thyroglobulin antibodies were present in titres of ≥1 25 in 15 of 36 and microsomal antibodies (PH) in titres of ≥1 10^5 in 28 of 34 subjects

Associated diseases

The concomitant diseases are listed in Table 5

A slightly hyperthyroid girl was examined by an ophthalmologist because of mild exophthalmus A clear papilloedema was observed and X ray films of the skull revealed close coronary and sagittal sutures and signs of intracranial pressure

Diabetes mellitus preceded thyrotoxicosis in all four patients

Many of our patients and their families had severe emotional problems The significance of this finding is difficult to evaluate because we have no control population Five patients needed psychotherapy

The result of gastric investigations will be published separately (10)

DISCUSSION

Our approach to therapy has been primarily conservative We have employed combined therapy with satisfactory results (4, 6) In one case, however, continuous growth of the thyroid could not be controlled The second choice of treatment has been subtotal thyroidectomy Patients with relapse after surgery or unsuitable for operation have been treated with radioiodine

Our remission rate of medically treated patients (44%) is comparable with that (40%) of the series of Vaidya et al (18), but is lower than the 61% achieved by Barnes & Blizzard (1) We did not find a decreasing remission rate as in the latter series On the contrary our remission rate is better than the 35% in our earlier study, but the difference is not statistically significant (13)

In half the patients subtotal thyroidectomy was considered necessary for various reaso[ns] to achieve lasting remission This equals t[he] role of surgery in the series of Vaidya et a[l] (56%) (18) and Barnes & Blizzard (48%) (In our earlier series subtotal thyroidecto[my] was performed in 63% of the patients f[ol]lowed (13) The decreased need for surge[ry] is presumably due both to the disappearan[ce] of endemic goitre in Finland (11) and to a d[e]crease of nodular goitres in our patients [In] our former series thyroid nodules occurred [in] 55% and in the present seires in 28% of t[he] patients In none of the patients with palpab[le] thyroid nodule(s) could a lasting remission [be] achieved with medical therapy This confir[ms] our earlier opinion that the presence of thyro[id] nodules indicates surgical treatment The fin[d]ing of papillary carcinoma in one of the nod

Table 5 *Concomitant problems in 40 childre[n] and adolescents with hyperthyroidism*

Disease	No of patients
Diabetes mellitus	4
IgA deficiency	2
Craniosynostosis	2
Idiopathic scoliosis	2
Hydrocephalus	1
Microcephalus	1
Atopic disease	2
Epilepsy	2
Severe emotional problems	5

Acta Pædiatr Scand 69 143–149, 1980

EVALUATION OF MECHANICAL VENTILATION IN NEWBORN INFANTS

I Techniques and survival rates

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ABSTRACT Lindroth, M, Svenningsen, N W, Ahlstrom, H and Jonson, B (Neonatal Unit, Department of Paediatrics, University Hospital, Lund, Sweden) Evaluation of mechanical ventilation in newborn infants I Techniques and survival rates Acta Paediatr Scand, 69 143, 1980.—The short term outcome with survival rate, causes of death and neonatal complications in a 6-year material comprising 253 infants treated with intermittent positive pressure ventilation (IPPV) in the neonatal period has been analyzed in relation to different primary disorders necessitating IPPV treatment. The total survival rate was 53% For the different diagnoses the survival rates were hyaline membrane disease (HMD) 41%, apnoea repetens of immaturity 85%, severe birth asphyxia 46% and septicemia 59% The total rate of pneumothorax during IPPV was 15% but occurred more often in the HMD group (28%) Trends in survival rates over the study period are discussed as are measurements for improvements

KEY WORDS Newborn infants, IPPV

Since the late nineteen sixties intermittent positive pressure ventilation (IPPV) for respiratory insufficiency in newborn infants has become a routine procedure in most neonatal intensive care units (4 6 11, 12) The decision to start IPPV involves weighing the immediate risk of death or damage caused by expectancy against the short term and long term risks involved in mechanical ventilation The optimal time for starting IPPV must be taken into consideration as well as the risks of transport to a neonatal intensive care unit with facilities for IPPV

The goal of the present paper is to describe IPPV techniques and survival rates in relation to various diseases in a material of infants treated with IPPV in the newborn period Pulmonary and neuro-developmental sequelae have been analyzed in a second paper (9)

PATIENTS AND METHODS

From January 1971 to December 1976 IPPV was given to 253 newborn infants in the Neonatal Intensive Care Unit (NICU) of the University Hospital in Lund—139 delivered in the Maternity Hospital in Lund and 114 admitted from other hospitals within the region The transport distances [illegible]

[illegible] 1501 g

All babies were nursed in incubators On admission umbilical arterial and venous catheters were inserted if possible and their positions controlled [illegible]

[illegible] glucose solutions The initial caloric supply of 60 kcal per kg body weight was gradually increased up to 120 kcal per kg at two weeks of age If these amounts could not be obtained with peroral breastmilk feeding total or supplemental parenteral nutrition was given with aminoacids (Vamin® Vitrum AB) glucose and fat (Intralipid®, Vitrum AB)

The patients were constantly supervised by a nurse during the intensive care period Continuous monitoring of respiration and heart rate was performed with a Saab Respimeter or Hewlett Packard cardiorespirograph Chest X-ray was taken on arrival after starting IPPV and thereafter about every 4–5th day Infections were treated

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Supported by grants from the Swedish Medical Research Council (No 29X-4732 and 14X-02872) Malmohus County Research Council and the Swedish National Association against Chest and Heart Diseases

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Submitted June 18, 1979
Accepted Aug 22, 1979

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Acta Pædiatr Scand 69 143–149, 1980

EVALUATION OF MECHANICAL VENTILATION IN NEWBORN INFANTS

I *Techniques and survival rates*

M LINDROTH N W SVENNINGSEN H AHLSTRÖM and B JONSON

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KEY WORDS Newborn infants, IPPV

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The goal of the present paper is to describe IPPV techniques and survival rates in relation to various diseases in a material of infants treated with IPPV in the newborn period Pulmonary and neuro-developmental sequelae have been analyzed in a second paper (9)

PATIENTS AND METHODS

was given to
ve Care Unit
139 delivered

in the Maternity Hospital in Lund and 114 admitted from other hospitals within the region The transport distances varied from 20 to 260 km The male-female ratio was 1.5 1 Among the 253 ventilated patients 126 were very low birth weight (VLBW) infants with birth weight less than 1501 g

All babies were nursed in incubators On admission umbilical arterial and venous catheters were inserted if possible and their positions controlled radiographically Prior to IPPV oxygen was administered in concentrations necessary to keep arterial pO_2 at 7–12 kPa Metabolic acidosis was corrected with i v infusions of sodium bicar
pply
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unts
ing,
total or supplemental parenteral nutrition was given with aminoacids (Vamin® Vitrum AB) glucose and fat (Intralipid® Vitrum AB)

The patients were constantly supervised by a nurse during the intensive care period Continuous monitoring of respiration and heart rate was performed with a Saab Respimeter or Hewlett Packard cardiorespirograph Chest X-ray was taken on arrival after starting IPPV and thereafter about every 4–5th day Infections were treated

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with ampicillin, cephalosporin or gentamycin, alone or in combinations in case of septicemia

The parents were encouraged to visit their baby daily or as often as possible, and also to take an active part in the nursing care, e g with gastric tube feeding, even during the intensive treatment period.

Diagnostic groups. The patients were placed in one of five diagnostic groups. Infants with more than one disease were classified according to the disease requiring IPPV treatment

The diagnostic groups were: 1) *Hyaline membrane disease (HMD)*, equivalent to idiopathic respiratory distress syndrome. *Diagnostic criteria* Within 8 hours of age at least two progressive clinical symptoms of grunting, intercostal retraction, tachypnoea or cyanosis *and* chest X-ray with reticulo granular pattern or airbronchogram and opaque lung fields, *and* increased right to left shunt according to Gupta et al. (7).

2) *Apnoea repetens (AR) Diagnostic criteria* Respiratory insufficiency with recurrent apnoea and bradycardia several hours or days after birth, in infants usually with gestational age of 32 weeks or less and with normal chest X-ray (3)

3) *Postasphyxia syndrome. Diagnostic criteria* Severe intra- or extra-uterine asphyxia with persistent foetal circulation and sometimes complicated by massive meconium aspiration Cyanosis, large right to-left shunting and severe acidosis were present (5)

4) *Septicemia Diagnostic criteria* Clinical and laboratory signs of septicemia and positive blood culture

5) *Other conditions* E g congenital malformations and hydrops foetalis

Indications for IPPV were recurrent apnoea with bradycardia, persistent severe hypercarbia and/or persistent severe hypoxia in high inspired oxygen concentration

IPPV techniques Naso-tracheal intubation with Portex-tubes No 2 5–3 0 was used in all cases Tubes were usually changed every 5–7 days in infants requiring prolonged IPPV treatment No infant was tracheostomized The ventilators used were Loosco Amsterdam Ventilator Mk 1 and Mk 2 (Loos & Co , Amsterdam, The Netherlands) or the Servo Ventilator 900 (Siemens-Elema AB Solna, Sweden) The latter can be set to produce constant pressure generated ventilation, which was frequently applied

For HMD and postasphyxia syndrome we started with inspiratory pressure of 20–25 cm H_2O, positive end-expiratory pressure (PEEP) of 2–5 cm H_2O, inspiration expiration ratio 1 1 and inspired oxygen concentration, FiO_2, of 40% For AR and septicemia we started with inspiratory pressure of 10–15 cm H_2O, zero PEEP, inspiration expiration ratio 1 1 or less, and 21–30% inspired oxygen The initial frequency was set at 40 per minute in all but few cases No sedatives were given routinely

Within 30–60 min blood gases were analysed to control the adequacy of the ventilatory settings The commonest corrections made were 1) for isolated hypercarbia an increase in frequency, 2) for isolated hypoxia an increase in FiO_2 and/or an increase in PEEP up to 8 cm H_2O, 3) for combined hypercarbia and hypoxia an increase in peak

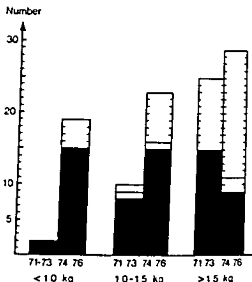


Fig 1 Number and outcome of IPPV treated infants wi hyaline membrane disease according to birth weight du ing 1971–73 and 1974–76. Black areas represent dead p tients and grey areas postneonatal deaths

pressure with or without simultaneous increase in fr quency, FiO_2 and/or PEEP Hypocarbia and hyperox were managed in the opposite directions Every change i the settings was tested with control blood gas analysi within 30–60 min

Since 1973 the infants were weaned from ventilato [illegible]

of recurrent apnoea or atelectasis of the upper right pul monary lobe However, no infant was kept on prolonged intubation after IPPV treatment

Physiotherapy and tube suction were performed rou [illegible]

spectively, were accepted as significant

RESULTS

Survival rates and causes of death The total survival rate was 53% It was the same for patients born in Lund and referred patients, and for boys and girls For VLBW infants, below 1501 g, it was 51% The total survival rate rose from 43–48% the first two years to 52–60% thereafter (Table 1). The lower survival rate for 1976 was partly due to 5 nonsurvivors started on IPPV who later proved to have severe lethal malformations. In com-

ble 1 *Outcome year by year of patients treated with IPPV and number of deaths not treated h IPPV*

D congenital heart disease

	IPPV treated				Dead without IPPV	
	Mean birth weight		Survivors			
ar	(kg)	N	N	%	CHD	Non-CHD
71	2.0±0 9	28	12	43	15	24
71	1 9±0 7	33	16	48	8	9
73	1 8±0 8	27	15	56	10	6
74	1 7±0 8	49	27	55	10	6
75	1 7±0 8	62	37	60	9	3
76	1 7±0 8	54	28	52	8	2
otal		253	135	53	60	50

parison this occurred in 3 dead patients during he whole previous 5-year period After 1974 nly a few infants died without IPPV apart rom patients with non viable congenital heart diseases

Survival rate was highest in AR patients (84%) in spite of their much lower birth weight (Table 2) Maturity was related to the outome only in HMD infants In this group the dead infants had significantly lower birth weight and gestational age ($p<0\ 01$) The survivival rates in the HMD and postasphyxia syndrome groups were 41% and 46% respectively However in 57 infants belonging to either of these groups and requiring very early IPPV *i e within 6 hours after birth*, the survival rate was only 27%

In the five diagnostic groups the following results were found (For comparison the material was divided into 2 periods I (1971–73) and II (1974–65))

HMD There were 37 IPPV patients during period I and 71 during period II (Fig 1) The mean birth weight decreased year by year from 1 8 to 1 3 kg In spite of this the total survival rate increased from 32% to 45% This increase was mainly caused by better results in infants weighing more than 1 0 kg Major causes of death were intraventricular hemorrhage (IVH) (*54*), septicemia (*6*), pneumonia (*3*) and pneumothorax (*3*) HMD was the only finding in 8 cases One infant died because of tracheal tube occlusion

AR Fig 2 shows that the number of AR patients more than doubled during period II The mean birth weight did not change The survival rate was 89% and 81% for period I and II, respectively Causes of death were

Table 2 *Gestational age (G a) and birth weight (B w) in different diagnostic groups for IPPV treated survivors and dead (mean values standard deviation and number of cases)*

	Total	Survivors		G a (weeks)		B w (kg)	
Diagnosis	(N)	N	%	Survivors	Dead	Survivors	Dead
HMD	108	44	41	32 8±3 0	31 1±3 1**	1 8±0 6	1 4±0 5**
AR	64	54	84	29 6±1 9	29 0+1 2	1 3±0 3	1 2±0 3
Asphyxia	37	17	46	38 0±3 8	38 9±2 7	3 2±0 8	3 0±0 7
Septicemia	27	16	59	32 4±2 5	32 1±3 9	1 7±0 5	1 5±0 6
Others	17	4	24	36 3±2 6	35 3±4 4	2 7±0.5	2 4±1 0
Total	253	135	53				

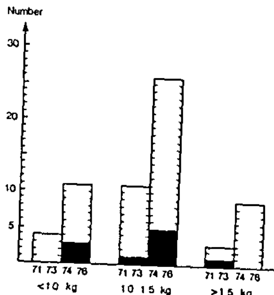


Fig 2 Number and outcome of IPPV treated infants with apnoea repetens according to birth weight during 1971–73 and 1974–76

BPD, pulmonary hemorrhage and septicemia in 2 cases each, IVH, *pneumothorax*, atelectasis and anoxic encephalomalasia in one case each

Postasphyxia-syndrome necessitating IPPV did not increase over the years (Fig 3) Only 8 of 37 infants were preterm, i e had a gestational age of less than 37 weeks Survival rate was 44% for period I and 47% for period II Major causes of death were IVH (7), anoxic encephalomalacia (4), aspiration pneumonia (4), disseminated intravascular coagulation or pulmonary haemorrhage (3), septicemia and cardiac tamponade in one case each

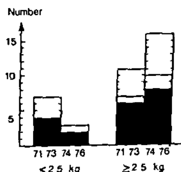


Fig 3 Number and outcome of IPPV treated infants with postasphyxia syndrome according to birth weight during 1971–73 and 1974–76

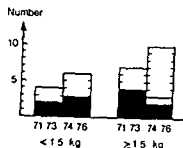


Fig 4 Number and outcome of infants treated with IPPV because of septicemia according to birth weight during 1971–73 and 1974–76

Septicemia as indication for IPPV occurred in one fullterm and 26 preterm infants Survival rate rose from 45% for period I to 69% for period II (Fig 4) Septicemia was the cause of death in 8 cases, IVH in 2 and disseminated intravascular coagulation in one case

Other conditions Only 4 of 17 infants in this group survived Causes of death were severe malformation in 8, large intracranial haemorrhage in 3 and hydrops foetalis in 2 cases

Late postneonatal deaths, i e infants who died after 2 months of age and after successful weaning from ventilator occurred in 8 cases—4 infants belonging to the HMD, 3 to the postasphyxia-syndrome and 1 to the septicemia diagnostic groups (Table 3) Five children with severe brain damage died in aspiration pneumonia, 1 patient with cardiac incompensation due to persistent ductus arteriosus died of infectious pneumonia and 2 deaths were due to sudden infant death syndrome at home

Neonatal complications Pneumothorax occurred during IPPV in 37 infants (15%) and was the probable cause of death in 5 cases Two of these also had pneumopericardium Pneumothorax occurred almost exclusively in the HMD (30 cases=27%) or postasphyxia syndrome groups (5 cases=14%) One 49 day old infant with HMD died at night because of an unrecognized tube occlusion At autopsy a large *intraventricular haemorrhage of old* date was also found We believe that this is the only case in which an infant died because

Table 3 *Clinical data and autopsy findings in late postneonatal deaths*

No	Original diagnosis	Duration of IPPV (days)	Sequelae	Age at death	Cause of death—autopsy findings
1	Preterm *HMD*	8	0	3 5 months	SIDS Slight airways infection
2	Preterm HMD	25	0	4 months	SIDS Slight BPD Signs of encephalomalasia in the left hemisphere
3	Preterm *HMD*	45	0	5 months	Pneumonia PDA Moderate BPD
4	Preterm *HMD* Cerebral haemorrhage	14	Retardation Convulsions	4 months	Pneumonia Microcephalus Subcortical encephalomalasia
5	Preterm *postasphyxia syndrome*	29	Retardation Convulsions	3 months	Pneumonia Microcephalus Subcortical encephalomalasia Slight BPD
6	Term *postasphyxia syndrome*	26	Retardation Convulsions	4 months	Pneumonia Cortical and subcortical encephalomalasia
7	Term *postasphyxia* syndrome	5	Retardation Convulsions	8 months	Pneumonia Microcephalus Subcortical encephalomalasia
8	Preterm *septicem a* meningitis	11	Retardation Infantile spasm	4 years	Pneumonia Hydrocephalus Only rests of remaining grey and white matter

HMD=Hyaline Membrane Disease PDA=Persistent Arterial Ductus BPD Bronchopulmonary dysplasia SIDS Sudden Infant Death Syndrome

of technical malfunction in the IPPV system In spite of prolonged intubation none of the survivors showed signs of tracheal stenosis In one dead patient a tracheal ulcer was found at autopsy

DISCUSSION

During a 6-year period certain changes in a clinical material and routines are inevitable In the present material the number of ventilated infants doubled during the last 3 years This increase was mainly due to a larger number of VLBW infants admitted to the NICU especially from other hospitals Another important factor was a more aggressive attitude towards the use of intensive care This has led to increased facilities e g more ventilators allowing us to treat all infants with any prospect of survival Thus 65% of the VLBW infants were treated with IPPV the last 3 years compared to 40% previously The percentage of VLBW infants surviving without IPPV was still unchanged (32%) In later years the major restrictions for IPPV have been confined to non treatable congenital malformations and severe perinatal cerebral damage due to prolonged asphyxia

After 1974, HMD infants were treated with early applied face chamber CPAP (2) This treatment has generally reduced the need for IPPV in HMD, but to a lesser degree in VLBW infants and referred patients arriving in a poor condition (17) A 4 fold increase of referred HMD patients to our NICU was the main cause of the increased use of IPPV in this group The survival rate increased from 32 to 45% because of better results in infants above 1 0 kg Further improvement of survival rate in HMD patients may be possible by the use of a specialized transport team with facilities for applying CPAP and IPPV during transport (12)

The larger number of IPPV treated AR patients during the last 3 years is explained by more liveborn immature babies both in Lund and the referral hospitals The survival rate of AR patients was 80 to 90% during the whole study period Six of the 10 non survivors died from pulmonary complications that could have a causal relation to IPPV It is therefore im

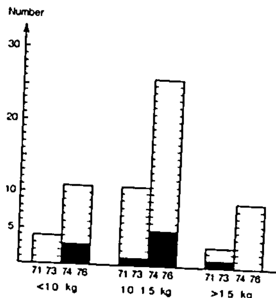


Fig 2 Number and outcome of IPPV treated infants with apnoea repetens according to birth weight during 1971–73 and 1974–76

BPD, pulmonary hemorrhage and septicemia in 2 cases each, IVH, pneumothorax, atelectasis and anoxic encephalomalasia in one case each

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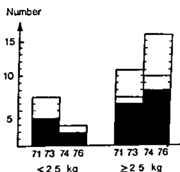


Fig 3 Number and outcome of IPPV treated infants with postasphyxia syndrome according to birth weight during 1971–73 and 1974–76

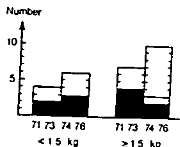


Fig 4 Number and outcome of infants treated with I[...] because of septicemia according to birth weight du[...] 1971–73 and 1974–76

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Submitted Febr 8 1979
Accepted Aug 20 1979

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portant to restrict IPPV in this group as far as possible. This can partly be achieved by applying low pressure CPAP (15) Since 1976 we have done this with the face chamber technique (2), thereby avoiding IPPV in several AR patients In recent years we have also tried theophyllamine in AR patients but several of these still needed low pressure CPAP or IPPV Over the years we have observed that the incidence of recurrent apnoea in AR patients can be reduced by adjusting accurately the inspired oxygen concentration The arterial P_{O_2} level appears to be a critical factor in the regulation of ventilation in VLBW infants (1, 13) It should be possible to avoid hyperoxia causing retrolental fibroplasia or hypoxia with brain damage by continuous transcutaneous P_{O_2} measurements (1, 15) Since 1976 the number of infants with AR requiring IPPV treatment has consequently become appreciably lower in our NICU

In the postasphyxia syndrome group the mortality rate, including late deaths, was 62%, with no change over the years In order to improve the prognosis in this group early diagnosis of intra uterine asphyxia is necessary, e g on the basis of cardiotocographic foetal heart rate and foetal scalp pH monitoring during delivery We are at present evaluating a terapeutic sheme including IPPV, steroid treatment of cerebral oedema and phenobarbital to lower cerebral metabolism and avoid convulsions after severe birth asphyxia (18)

Most infants with postasphyxia syndrome who died in the present material—including the 3 cases of late deaths—had no spontaneous breathing within 30–60 min after delivery At present we start IPPV only in those infants with severe birth asphyxia who are breathing spontaneously within 45 min of intensive resuscitation

In septicemia the increasing survival rate from 45 to 69% was partly due to the earlier diagnosis and treatment of this disease This also seemed to reduce the need from IPPV in these cases

The intensive care with IPPV treatment of VLBW infants is currently under debate (8, 11 14, 16) The survival rate for AR patients even with a birth weight below 1 0 kg was high in our material For HMD patients the results were less satisfactory for the smallest infants, whereas more infants with birth weight above 1 0 kg survived later in the study period An active approach towards VLBW infants seems well justified in neonatal intensive care units with the necessary facilities and experience Such an approach is not only immediately rewarding but is also a prerequisite of further progress The low incidence of late sequelae in this material (9) further supports an active attitude to the management of VLBW infants

The psychosocial aspects of active family support during IPPV treatment of newborn infants are of utmost importance During the intensive care period we inform the parents every day about their baby's progress and we stimulate them to visit and take an active part in the nursing care We agree with Kopelman (8) that this is a most important part of the neo natal intensive care for the future development of the immature very sick newborn infant

Finally, a successful application of neonatal IPPV treatment is not only related to IPPV technique per se but also to education, constant training and devotion of all personnel involved in the intensive care of the newborn infant (8, 14) The survival rate and long term outcome of neonatal IPPV therapy depends upon the ability to cope with both technical and psychological problems

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Submitted Febr 8 1979
Accepted Aug 20 1979

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Acta Pædiatr Scand 69 151–158, 1980

EVALUATION OF MECHANICAL VENTILATION IN NEWBORN INFANTS

II *Pulmonary and neuro-developmental sequelae in relation to original diagnosis*

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ABSTRACT Lindroth, M, Svenningsen, N W, Ahlström, H and Jonson, B (Neonatal Unit, Department of Paediatrics, University Hospital, Lund, Sweden) Evaluation of mechanical ventilation in newborn infants II Pulmonary and neuro-developmental sequelae in relation to original diagnosis Acta Paediatr Scand, 69 151, 1980.—The incidence of bronchopulmonary dysplasia (BPD) and neuro-developmental sequelae in 135 infants surviving intermittent positive pressure ventilation (IPPV) in the newborn period were studied in relation to primary disorders requiring IPPV The rate of BPD increased over the 6-year study period in hyaline membrane disease survivors from 14% to 28%, but decreased in infants with apnoea repetens from 38% to 13% Immaturity seemed to be one important factor for development of BPD The incidence of neuro-developmental sequelae in IPPV treated infants fell from 22% to 13% over the years In infants with birth weight below 1501 g the rate of neurological handicaps was 11%

KEY WORDS Newborn infants, IPPV, neuro-developmental follow up study, pulmonary mechanics bronchopulmonary dysplasia

During the last decades intermittent positive pressure ventilation (IPPV) has been widely used in newborn infants with respiratory insufficiency The extension of IPPV to include even infants below 1 0 kg and the increasing survival rates have caused apprehension about a possible increased number of survivors with respiratory and neurological sequelae (4, 19) The aim of this study has been to evaluate the frequency of bronchopulmonary dysplasia and major neuro-developmental sequelae in relation to original diagnosis and birth weight in a material of infants treated with IPPV in the neonatal period described in a previous paper (11)

PATIENTS AND METHODS

During 6 years (1971–1976) 253 infants were treated with IPPV in the Neonatal Intensive Care Unit in Lund Of these 139 were [illegible] and 114 referred from other hospitals Half of the patients (126) were very low birth weight (VLBW) infants with a birth weight below 1 501 g

The patients were placed in one of five diagnostic groups Hyaline membrane disease (HMD) Apnoea repetens (AR) Postasphyxia syndrome Septicemia or Other conditions" Definitions of the diagnoses indications for IPPV and details of the IPPV treatments have been presented separately (11)

The diagnosis bronchopulmonary dysplasia (BPD) was based on stage III or IV chest X ray changes according to Northway et al (15) In non survivors the diagnosis was verified at autopsy which was performed in all infants who died

Follow-up studies The following studies were made before discharge from the NICU

1 Ophthalmological and neurological examination (including sonoencephalogram and electroencephalogram in

Supported by grants from the Swedish Medical Research Council (No 29X 4732 and 14X-02872) Malmohus County Research Council and the Swedish National Association against Chest and Heart Diseases

ble 1 *Clinical data and autopsy findings in 10 non surviving infants with bronchopulmonary splasia*

D Atrial Septal Defect IVH Intraventricular Haemorrhage PDA=Persistent Ductus Arteriosus SIDS=Sudden ant Death Syndrome

Case	Original diagnosis	Birth weight (kg)	Gestational age (weeks)	Duration IPPV (days)	Age at death (months)	Cause of death—autopsy findings
rly deaths						
	HMD	0 8	26	16	2*	Septicemia—Marked BPD
	HMD	0 6	27	15	1/2	Heart failure—ASD Moderate BPD Subcortical encephalomalasia
	HMD	1 2	30	47	1 5	Tube occlusion—Moderate BPD Large old IVH Subcortical encephalomalasia
ı	AR	0 9	29	44	1 5	Septicemia—Marked BPD Subcortical encephalomalasia
ı	AR	1 4	29	50	9 5*	Right heart failure—Marked BPD Cortical and subcortical encephalomalasia
5	AR	1 0	29	20	1	Right heart failure—Marked BPD
7	Intracranial haemorrhage	1 2	28	30	1	Septicemia—Moderate BPD Hydranencephalia
ate deaths						
8	HMD	1 5	29	43	5	D
9	HMD	1 3	33	25	4	
0	Postasphyxia syndrome	2 6	36	29	3	

Always oxygen dependent and intermittently on CPAP or IPPV treatments for long periods

repetens (AR) Two cases had the primary diagnoses intraventricular hemorrhage and postasphyxia syndrome (Cases 7 and 10, Table 1)

As shown in Table 1 10 BPD infants died Patients No 1–7 died during IPPV treatment whereas patients No 8–10 died after successful weaning from IPPV In these latter cases the causes of death were not directly related to BPD why they were assigned to BPD survivors

The rates of BPD in the HMD and AR groups were 13 and 22% respectively (Table 2) Comparing the two 3 year periods (1971–73 and 1974–76) the incidence of BPD in HMD survivors increased from 14 to 28% but decreased in AR survivors from 38 to 13% These changes were statistically not significant

Within both the HMD and AR groups the patients developing BPD had significantly lower gestational age and birth weight (Table 3) Furthermore, the duration of IPPV was appreciably longer in BPD patients as shown in Table 4 and Fig 5 For non surviving BPD patients the duration of IPPV ranged from 15 to 50 days (Table 1) However, in HMD and AR patients without BPD the duration of IPPV was inversely correlated to gestational age ($r = -0\ 39^{**}$ in HMD and $-0\ 47^{***}$ in AR) (Fig 5)

Table 2 *Incidence of bronchopulmonary dysplasia (BPD) after neonatal IPPV in patients with hyaline membrane disease (HMD) or apnoea repetens (AR)*

Diagnosis	Survivors	Dead	Total	Incidence (%)
HMD	11/44	3/64	14/108	13
AR	11/54	3/10	14/64	22
Total	22/108 20%	6/74 8%	28/172	16

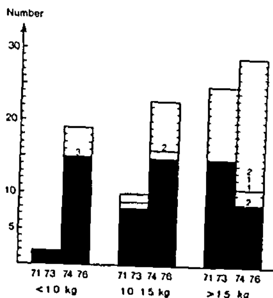


Fig 1 Number and outcome of IPPV treated *infants with* hyaline membrane disease according to birth weight during 1971–73 and 1974–76 Black areas represent dead patients and grey areas postneonatal deaths The types of sequelae are numbered as follows 1 Diplegia, 2 Retardation, 3 Retrolental fibroplasia, 4 Hemiplegia, 5 Tetraplegia, 6 Athetosis, 7 Hydrocephalus

All patients were examined every third month with neuro-developmental evaluation in our special follow up clinic by two of us (N S or M L) Some referred patients were similarly followed up in their local children s hospital All infants were followed up to at least 14 *months* of age Thereafter the normal children were controlled within the Child Health Services once a year Infants

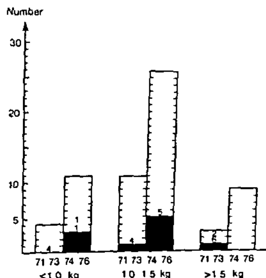


Fig 2 Number and outcome of IPPV treated infants with apnoea repetens according to birth weight during 1971–73 and 1974–76

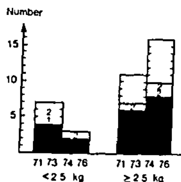


Fig 3 Number and outcome of IPPV treated infants w postasphyxia syndrome according to birth weight dur 1971–73 and 1974–76

Since 1975 all patients were tested with free field a diometry, and those with suspect findings were test further with brain stem audiometry or electrical respo audiometry Ophthalmological examination was repea in infants with abnormalities, e g strabismus

Statistical methods The statistical methods used we chi square with Yate s correction and Student s *t* test examine differences between groups Probability values $p<0.05$ and $p<0.01$, indicated in the tables as * and * respectively, were accepted as significant

RESULTS

Survival rate As previously reported 135 o the 253 IPPV treated infants survived th neonatal period, but 8 infants died postneona tally after 2 months of age. These late death have been presented in detail in our previou report (11) The number of survivors and earl and late deaths in the different diagnosti groups are shown in Figs 1–4

Bronchopulmonary dysplasia Bronchopul monary dysplasia (BPD) was diagnosed in 3(patients It occurred in 14 patients with hyaline membrane disease (HMD) and 14 with apnoea

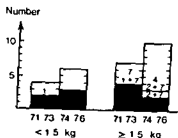


Fig 4 Number and outcome of infants treated with IPPV because of septicemia according to birth weight during 1971–73 and 1974–76

Table 1 *Clinical data and autopsy findings in 10 non-surviving infants with bronchopulmonary dysplasia*

ASD=Atrial Septal Defect IVH=Intraventricular Haemorrhage, PDA=Persistent Ductus Arteriosus, SIDS=Sudden Infant Death Syndrome

No	Original diagnosis	Birth weight (kg)	Gestational age (weeks)	Duration IPPV (days)	Age at death (months)	Cause of death—autopsy findings
Early deaths						
1	HMD	0 8	26	16	2*	Septicemia—Marked BPD
2	HMD	0 6	27	15	1/2	Heart failure—ASD Moderate BPD Subcortical encephalomalasia
3	HMD	1 2	30	47	1 5	Tube occlusion—Moderate BPD Large old IVH Subcortical encephalomalasia
4	AR	0 9	29	44	1 5	Septicemia—Marked BPD Subcortical encephalomalasia
5	AR	1 4	29	50	9 5*	Right heart failure—Marked BPD Cortical and subcortical encephalomalasia
6	AR	1 0	29	20	1	Right heart failure—Marked BPD
7	Intracranial haemorrhage	1 2	28	30	1	Septicemia—Moderate BPD Hydranencephalia
Late deaths						
8	HMD	1 5	29	43	5	Pneumonia, Heart failure—PDA Moderate BPD
9	HMD	1 3	33	25	4	SIDS—Slight BPD Signs of encephalomalasia in the left hemisphere
10	Postasphyxia-syndrome	2 6	36	29	3	Aspiration—Microcephalus Slight BPD Subcortical encephalomalasia

* Always oxygen dependent and intermittently on CPAP or IPPV treatments for long periods

repetens (AR) Two cases had the primary diagnoses intraventricular hemorrhage and postasphyxia syndrome (Cases 7 and 10, Table 1)

As shown in Table 1 10 BPD infants died Patients No 1–7 died during IPPV treatment whereas patients No 8–10 died after successful weaning from IPPV In these latter cases the causes of death were not directly related to BPD why they were assigned to BPD survivors

The rates of BPD in the HMD and AR groups were 13 and 22%, respectively (Table 2) Comparing the two 3 year periods (1971–73 and 1974–76) the incidence of BPD in HMD survivors increased from 14 to 28% but decreased in AR survivors from 38 to 13% These changes were statistically not significant

Within both the HMD and AR groups the patients developing BPD had significantly lower gestational age and birth weight (Table 3) Furthermore, the duration of IPPV was appreciably longer in BPD patients as shown in *Table 4 and Fig 5* For non-surviving BPD patients the duration of IPPV ranged from 15 to 50 days (Table 1) *However, in HMD* and AR patients without BPD *the duration of* IPPV was *inversely correlated* to gestational age ($r=-0\ 39^{**}$ *in HMD* and $-0\ 47^{***}$ in AR) (Fig 5)

Table 2 *Incidence of bronchopulmonary dysplasia (BPD) after neonatal IPPV in patients with hyaline membrane disease (HMD) or apnoea repetens (AR)*

Diagnosis	Survivors	Dead	Total	Incidence (%)
HMD	11/44	3/64	14/108	13
AR	11/54	3/10	14/64	22
Total	22/108 20%	6/74 8%	28/172	16

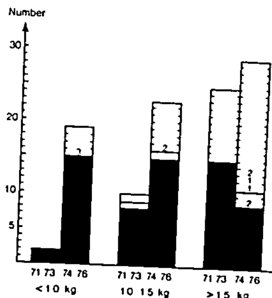


Fig 1 Number and outcome of IPPV treated infants with hyaline membrane disease according to birth weight during 1971–73 and 1974–76 Black areas represent dead patients and grey areas postneonatal deaths The types of sequelae are numbered as follows 1 Diplegia, 2 Retardation, 3 Retrolental fibroplasia, 4 Hemiplegia, 5 Tetraplegia, 6 Athetosis, 7 Hydrocephalus

All patients were examined every third month with neuro-developmental evaluation in our special follow up clinic by two of us (N S or M L) Some referred patients were similarly followed up in their local children s hospital All infants were followed up to at least 14 months of age Thereafter the normal children were controlled within the Child Health Services once a year Infants assessed as psychomotor retarded were obviously late in their ability to sit, walk and talk, taking into consideration the degree of prematurity at birth

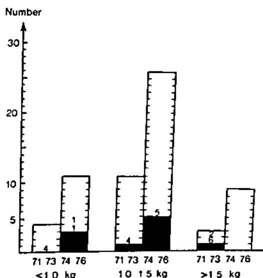


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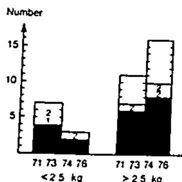


Fig 3. Number and outcome of IPPV treated infants with postasphyxia syndrome according to birth weight during 1971–73 and 1974–76

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Statistical methods The statistical methods used were chi square with Yate s correction and Student s *t* test to examine differences between groups Probability values $p<0.05$ and $p<0.01$, indicated in the tables as * and ** respectively, were accepted as significant

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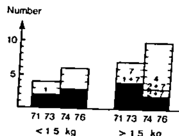


Fig 4 Number and outcome of infants treated with IPPV because of septicemia according to birth weight during 1971–73 and 1974–76



; 6 Dynamic compliance (log scale) in relation to ıgth in 21 infants with bronchopulmonary dysplasia : investigated infants are connected with lines The ıshed lines indicate the regression lines and ±95% con lence limits for infants below and over 50 cm length for ırmal infants (12)

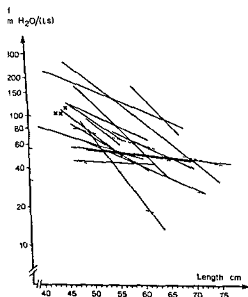


Fig 7 Pulmonary functional resistance (long scale) in relation to length in 21 infants with bronchopulmonary dysplasia Re-investigated infants are connected with lines The dashed lines indicate the regression line and ±95% confidence limits for normal infants (12) Notice that proper impression of very high resistance values at the first examination is lost because of the log scale

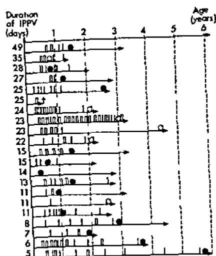


Fig 8 Bronchitis and latest chest X ray findings in 21 infants with bronchopulmonary dysplasia ●, normal chest X ray, Ø, radiographic residuals, ⊓, bronchitis or pneumonia treated in hospital, |, bronchitis treated at home

tients born during the first 3-year period (1971–73) was 9 of 41 (22%) compared to 11 of 86 (13%) born in 1974–76. There was no difference between patients born at this hospital and referred patients The neuro-developmental handicaps found in the different diagnostic groups are shown in Figs 1–4 In HMD and AR there was no correlation between low Apgar score and later sequelae The total rate of neuro developmental sequelae in the diagnostic groups were: HMD 10%, AR 13%, postasphyxia-syndrome 21%, septicemia 33% and 1 of 4 survivors in "other conditions" (Figs. 1–4)

The sequelae rate in the VLBW infants (less than 1501 g) treated with IPPV was 7 of 64 (11%) During the study period (1971–76) 71 VLBW infants survived without IPPV and 4 (6%) had neurological handicaps (3 diplegia and 1 spastic hemiplegia) The difference between ventilated and non-ventilated VLBW survivors was not statistically significant

IPPV was given to 39 infants below 1000 g and 16 (41%) survived Twelve of these (75%) survived without any neuro-developmental sequelae

Table 3. *Gestational age and birth weight in patients with and without bronchopulmonary dysplasia (BPD)*

	N	Gest age (weeks)	Birth weight (kg)
HMD			
No BPD	94	31 6±3 3	1 6±0 6
BPD	14	29 3±2 1*	1 2±0 4*
AR			
No BPD	50	29 8±1 7	1 3±0 3
BPD	14	28 6±1 6*	1 1±0 2*

* = significantly different from patients without BPD

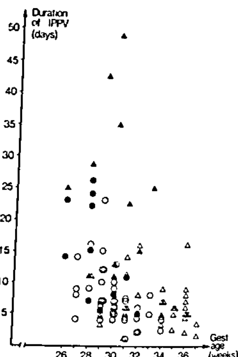


Fig 5 Bronchopulmonary dysplasia (BPD) in relation to duration of IPPV and gestational age in surviving infants treated for hyaline membrane disease (HMD) and apnoea repetens (AR) O, AR without BPD, Δ, HMD without BPD, ●, AR with BPD, ▲, HMD with BPD

The maximum inspired oxygen concentration during IPPV was 40 to 100% in 22 of the 30 BPD patients but less than 40% in 8 surviving BPD patients

Pulmonary mechanics were measured in 57 patients because of BPD, other chest X-ray changes or prolonged oxygen dependency One or more examinations were made in 20 of the 22 surviving BPD patients. Most of them had low dynamic compliance and/or high pulmonary resistance at the first investigation after IPPV. At re-examination during the first year of life there was a strong tendency to normalization of these parameters (Figs 6 and 7)

The clinical course and latest chest X-ray findings of 21 surviving BPD patients are illustrated in Fig 8 Most patients had recurrent bronchitis or pneumonia requiring treatment in hospital on one or more occasions However, recurrent bronchitis usually subsided within the first two years Chest X ray showed gradual improvement and becam normal within the first 2 years in 10 patien and later in 4 After 2 years of age 5 childre still had residual radiographic signs with minc streaks of fibrous character alternating wit small areas of overdistention However, onl 2 of these 5 infants still had recurrent bror chitis In general chest X-ray became norm: later than pulmonary mechanics

Table 4 *Duration of IPPV in surviving patients with and without bronchopulmonary dysplasia (BPD)*
Median and range

Diagnosis	BPD N	BPD IPPV (days)	No BPD N	No BPD IPPV (days)
HMD	11	22 5** (11–49)	33	5 (1 7–16)
AR	11	11** (5–26)	43	6 5 (1–23)
Total	22	15 (5–43)	76	6 (1–23)

** = significantly different from patients without BPD (Mann Whitney's Rank sum test)

Upper airway damage No infant in thi material was tracheostomized At autopsy one patient 4 days old treated 4 days with IPPV had a tracheal ulcer None of the survivors has shown symptoms of tracheal stenosis There was no case of necrosis of nasal septum or alae nasi

Neuro-developmental function At the latest follow-up examination 20 children (16%) showed neurological or developmental abnormalities (Table 5) The incidence in pa-

terial suffered from recurrent bronchitis en requiring hospitalization during the first ears

The absence of tracheal stenosis or other per airway complications in spite of intuba- in up to 49 days may seem unexpected owever, using pressure limited ventilation e have not aimed at tight fitting tracheal bes On the contrary, we have deliberately ed relatively small tubes allowing a leak, ossibly facilitating secretion drainage The nall tubes may have led to avoidance of a onstant pressure on the mucus membrane ausing ulceration and stenosis

Neuro developmental sequelae The bene- ts and hazards of intensive care, including PPV, must continuously be re evaluated pprehension has been expressed about the isk of an increasing number of handicapped nfants as the survival rate improves (4, 19) On the other hand several investigations have hown encouraging results even in very small nfants below 1000 g (10, 16, 21, 22) In the present material the incidence of neuro-devel- opmental sequelae after neonatal IPPV actual- ly declined from 22% in the first 3 year period to 13% during the following The total inci- dence of 16% was comparable to the findings of others (8, 9, 13) Like others we found no difference in survival or sequelae rate between patients born in Lund and patients referred from other hospitals (3, 13, 21) Two factors may have contributed to this 1) Many high- risk pregnant women were referred to our Maternity hospital prior to delivery so that these infants influenced the results of the pa- tients born in Lund 2) The survival rate for the referred infants could have been favoured by a selection by the referring hospital sending only those deemed "capable of surviving"

In the groups of postasphyxia-syndrome and septicemia the rates of sequelae were disap- pointingly high (see Figs 3 and 4) To avoid the risk of prolonged ventilator treatment of severely brain damaged infants, we now re- strict IPPV at severe perinatal asphyxia to infants with signs of spontaneous breathing within 45 min of resuscitation (11) For septi- cemia preventive measures, earlier diagnosis and intensive antibiotic treatment may reduce the sequelae rate

Very low birth weight infants The incidence or neurological sequelae in our IPPV treated VLBW infants was 11% which can be com- pared to 9% in the report by Stewart & Rey- nolds (21) and 29% reported by Fitzhardinge et al (6) However, certain infants considered brain damaged were taken off IPPV in the first mentioned study, whereas all patients in the latter were referred patients, often in bad condition on arrival.

In the present study there was no signifi- cant difference in the incidence of neurological sequelae between IPPV and non-IPPV treated VLBW infants This agrees with the results in Kamper s comparison of IPPV treated HMD infants with matched controls (9).

Our findings support the view that IPPV in the newborn period, including VLBW infants, does not necessarily increase the proportion of handicapped children For infants with a birth weight above 1 0 kg the cause of respiratory insufficiency seemed to be of greater prog- nostic value than birth weight. Also, as earlier mentioned, pulmonary sequelae with BPD occurring in immature infants had mostly a benign course

On the other hand, it is important to realize that IPPV treatment of VLBW infants implies at times prolonged ventilator treatment with a heavy load on the neonatal care unit It demands a specially trained intensive care staff available day and night throughout the year We therefore believe that this special type of neonatal intensive care should be con- centrated to regional centers with the neces- sary economic and personal resources

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Table 5. *Developmental and neurological sequelae after neonatal IPPV treatment in 127 survivors at 14 months to 7 years of age*

Diagnosis	*N*
Spastic diplegia	9
Spastic hemiplegia	3
Tetraplegia	1
Athetosis	1
Hydrocephalus	4
Psychomotor retardation	7
Total number of sequelae	25
Total number of patients with sequelae	20/127=16%

Retrolental fibroplasia was found in one HMD patient (birth weight 0 8 kg and gestational age 27 weeks) treated with IPPV for 25 days with maximum inspired oxygen concentration of 40–50% for 113 hours

Hearing defects were found in 2 children who both had severe postnatal jaundice requiring several exchange transfusions They also had spastic hemiplegia and athetosis

DISCUSSION

Bronchopulmonary dysplasia Several etiological factors may be involved in the development of BPD, e g treatment with IPPV, immaturity of the patient, duration of IPPV, primary disease, high oxygen concentration or high ventilatory pressures

The following observations made in our 30 BPD cases indicate possible connections of causes with BPD

All reports of BPD so far concern endotracheally intubated patients (2, 5, 7, 8, 15, 17, 18) Infants treated with CPAP via endotracheal tube can in exceptional cases develop BPD, whereas this never occurs with CPAP treatment without intubation, e g with the face chamber technique (2, 17) Thus IPPV seems to be a main factor of BPD with endo tracheal intubation as a possible additional factor

As shown in Fig 5 no infant of a gestational age above 33 weeks developed BPD This observation indicates that immaturity to an important causal factor As proposed others this may be caused by a higher susce bility of the immature lung tissue to ox (2, 5, 17) The importance of the dura of IPPV treatment is difficult to eval although it is conspicious that no infant trea for less than 5 days developed BPD Howe when BPD has developed, lung damage pe extends the duration of IPPV

BPD occurred in both HMD and AR tients Apnoea repetens (AR) in immature fants is probably not initially a lung disea This seems to contradict the proposal t BPD is only the end result of the prim disease (20)

Our results support the opinion that v high inspired oxygen concentrations are r necessary for the development of BPD (1 and are contrary to those of others who stre high oxygen as the main causal factor (5 15)

Reynolds & Taghizadeh (18) emphasi mechanical factors in IPPV treatment, e high airway pressures, as matters of prin importance Yet, in the present material BP developed also at low peak pressures, mo AR patients with BPD were ventilated wi peak pressures of 15 to 20 cm H_2O

A mechanical factor that we suggest shoul be further evaluated is uneven volumetric lun expansion leading to shear stresses within th lung tissue These stresses are caused by th interdependence of non-uniformly expande lung areas as described by Mead et al (14)

The falling BPD incidence over the years i our AR patients may reflect improved tech niques for maintenance of adequate ventila tion The increased rate in the HMD group is probably related to the fact that more imma ture HMD patients with severe disease sur vived in recent years

Two of the 30 patients with BPD died be cause of the lung damages In surviving BPD infants a favourable long-term prognosis has been reported (7, 8) We have made the same observations although many infants in our

Acta Pædiatr Scand 69 159–163, 1980

PULMONARY FUNCTION STUDIES IN LONG-TERM SURVIVORS WITH ARTIFICIAL VENTILATION IN THE NEONATAL PERIOD

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ABSTRACT. Borkenstein, J , Borkenstein M and Rosegger, H. (Department of Medicine and Department of Paediatrics, Pulmonary function studies in lo period Acta Paediatr Scand, 69 years who required intermitten. obtained The conditions necessitating artificial ventilation were hyaline membrane disease, neonatal apneic spells, aspiration of milk, and birth asphyxia On examination the probands were in a good healthy state and without any subjective signs of dyspnea Lung volumes could be measured in all of the probands They did not show any statistically significant deviation from standards for height and correlated with the age of the probands Time related flow rates were sufficiently measurable in 7 probands only, who cooperated adequately In 6 of them the expiratory flow showed a decrease of the effort independent portion with a slight increase in the total airway resistance There was no correlation between the condition requiring artificial ventilation, the former therapeutic characteristics and the degree of the pathological lung function tests The results of this investigation suggest damage of the smallest airways which could be the reason for the obstructively impaired expiratory patterns seen in 6 of the probands

KEY WORDS Newborn infants, artificial ventilation, long term prognosis, pulmonary function

Artificial ventilation of newborn infants suffering from various respiratory diseases such as idiopathic respiratory distress syndrome (IRDS), neonatal apneic attacks and birth asphyxia has undoubtedly reduced mortality and morbidity and improved the neurological outcome for these patients However, several investigators found a pathological lung function and an increased tendency to severe lower respiratory tract infections in long term survivors of IRDS, who were ventilated artificially The question still remains unsolved whether artificial ventilation of any kind in infants may cause permanent lung damage leading to chronic respiratory dysfunction in later life or only transitory destruction of the lung tissue with subsequent complete structural and functional repair As our patients were ventilated on different respirator settings for periods ranging from 1 to 624 hrs we looked for a possible correlation between severity of pulmonary changes and parameters of ventilator therapy including oxygen concentration

PATIENTS AND METHODS

Mechanical ventilation was introduced in the Neonatal Intensive-care Unit of the Pediatric University Hospital in Graz as a routine method for treatment of respiratory insufficiency at the end of 1972 Then only intermittent positive pressure ventilation (IPPV) was available because of technical reasons (Loosko Amsterdam infant respirator) but it was increasingly replaced by other methods over a period of about 2 years During this time 111 infants were artificially ventilated for respiratory insufficiency of varying origin From the 55 (50%) survivors, 11 patients (7 male, 4 female) were selected (Table 1) They were treated with IPPV only and at the time of further tests were old enough for cooperation (2½–5½ years, mean 3 9) They had required endotracheal intubation and IPPV for the following conditions Clinical and radiological signs of IRDS in cases 1, 3 7, 9 with initial

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Submitted Feb 8, 1979
Accepted Aug 20, 1979

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function tests

	Sex	Age (yrs)	TLC ml	TLC %	VC ml	VC %	FRC ml	FRC %	RV ml	RV %	RV/TLC	^{MEF}VC 50%/TLC	FEV$_1$ (% VC)	R_{tot}	%
1	f	3 5	1 150	109	900	110	500	116	250	106	21 7	0 8	66	8 8	105
2	m	2 5	810	107	640	110	350	116	170	99	20 9	–	–	–	–
3	m	4 5	1 350	93	1 050	94	500	83	300	83	22 2	1 1	70	7 0	96
4	m	4 7	1 260	89	960	88	550	94	300	95	23 8	–	–	–	–
5	m	5 4	1 650	111	1 300	113	600	97	350	106	21 2	1 0	69	7 7	105
6	m	4 2	1 600	113	1 250	114	600	102	350	111	21 8	0 6	68	7 5	101
7	m	3 8	1 550	119	1 250	123	550	101	300	103	19 3	–	–	–	–
8	f	4 0	1 260	96	960	96	500	92	300	103	23 8	0 8	63	8 1	107
9	m	5 1	1 200	85	900	82	550	94	300	95	25 0	0 7	68	7 7	104
10	f	3 8	950	90	700	86	450	104	250	106	26 0	0 8	70	8 4	100
11	f	3 5	1 050	103	800	101	450	108	250	109	23 8	–	–	–	–

probands at pulmonary function testing had no measurable influence on the results obtained. The blood gases (finger-pricks) were all within normal limits (see Table 4)

DISCUSSION

All children investigated in this study were ventilated during infancy for different periods, using different ventilator settings and different concentrations of ambient oxygen The normal static lung volumes, measured in all 11 probands, suggest a normal development of their lungs with respect to age and body size There are, however, obstructive inhomogeneities of the distribution of ventilation, which are age independent and therefore without correlation to the time interval between artificial ventilation and lung function testing.

There is no obvious correlation between the severity of the pathological test results and the former therapeutic management such as oxygen concentration, respirator setting and the length of ventilation. Pat. 5, who was ventilated only for 1 hr had a borderline ^{MEF}VC 50%/TLC, whereas pat 3, who was ventilated for 96 hrs, showed an entirely normal value Pat 10, who was ventilated for 624 hrs with

Table 3 *Duration of ventilation and highest ventilator settings*

including oxygen concentration recorded for at least 24 hrs indication for the onset of artificial ventilation, gestational age (GA) in weeks birth weight (BW) (g) and ^{MEF}VC 50 % which should exceed the value of 1

Proband	Hours IPPV	Peak pressure (hPa)	F/min	O_2%	Indication	GA	BW	^{MEF}VC 50%/TLC
1	40	31 4	50	50	HMD	35	2 210	0 8
2	120	26 5	50	75	Apnea	32	1 380	–
3	96	29 4	40	25	HMD	36	2 600	1 1
4	48	29 4	40	Air	Birth Asphyxia	42	4 100	–
5	1	19 6	30	Air	Apnea	39	2 900	1 0
6	275	29 4	40	Air	Apnea	31	1 280	0 6
7	165	29 4	40	35	HMD	34	2 200	–
8	34	19 6	40	24	Aspiration	36	1 900	0 8
9	67	19 6	45	35	HMD	32	1 650	0 7
10	624	34 4	40	24	Apnea	30	1 200	0 8
11	280	24 5	40	Air	Asphyxia	38	3 000	–

11 792872

Table 1 *Details of 11 probands mean values, lowest and highest value in parentheses*

Peak pressure, frequency and % O_2 indicate correspondingly maximal respiratory settings, which were applied for at least 24 hrs

Number of probands	11	(7 male, 4 female)
Age at pulmonary function testing (years)	3 9	(2 5–5 4)
Birth weight (g)	2 220±880	(1 200–4 100)
Gestational age (weeks)	35±3 7	(30–42)
Duration of artificial ventilation (hrs)	159	(1–624)
Peak pressure (hPa)	26 6	(19 6–34 3)
Frequency (c/min)	41 4	(30–50)
O_2 (%)	34 8	(21–75)

P_{aO_2} in 100% O_2 of 8 00 kilopascals or less before intubation, apneic spells of prematurity, lasting 20 sec or more followed by bradycardia of 80/min or less, not responding to cutaneous stimuli and vigorous suction of mouth, nose and pharynx in cases 2, 5, 6, 10, severe aspiration of milk in case 8, birth asphyxia with an Apgar score at 1 min of 3 or less at 5 min of 5 or less and without spontaneo s

without significant changes in this blood gases In the 11 patients the duration of ventilation ranged from 1–624 hrs (mean 159) the period of CPAP excluded Hypercapnia was treated by means of increasing frequency and/or inspiratory peak pressure keeping P_{aO_2} between 8 00–8 65 kPa The lowest possible pressure was chosen in order to avoid mechanical lung trauma The highest recorded peak pressures used for more than 24 hrs ranged from 19 6 to 34 3 hPa (mean 26 6) Some of the cases with IRDS required higher pressures for periods around 1 hr after intubation to correct the blood gases The frequency ranged from 30 to 50 cycles per min (mean 41 4) Inspired oxygen [illegible] 32 and [illegible] used in [illegible] 72 hrs (mean 34 7) The gas mixtures were humidified by means of ultrasonic humidifiers Special care of the patients included tube suction every hour rinsing of the endotracheal tube with small amounts of saline to prevent tube obstruction and chest physiotherapy Pulmonary complications during and after artificial ventilation were pneumonia in cases 1 2 and 6 and bronchopulmonary dysplasia stage 2 in case 10 The microbiological cultivation of tracheal secretions and endotracheal tubes revealed *Pseudomonas aeruginosa* in 5 cases (1 2 3 4 10) The neurological and statomotoric development of all infants investigated was normal except in case 8 who showed a transient minor handicap which subsided during her 4th year of life At the time of the examination all children were in a normal healthy condition

With a pneumotachograph and a whole body plethysmograph in the open system (Pulmostar, Fenyves & Gut Basle, Switzerland) the following values were measured total lung capacity (TLC) vital capacity (VC) functio residual volume (RV), maximal expiratory flow at 25 a 50% of VC (MEF_{25}, MEF_{50}) forced expiratory volume 1 sec (FEV_1) and the total airway resistance (R_{tot}) Me values from three subsequent measurements in each p tient were taken for further analysis and discussion T quotient RV/TLC was calculated, the maximal expirato flow in litres/sec referred to the volume of the TLC The values were expressed in % of the predicted values pu lished by Engstrom et al (3) and Weng & Levison (1 These authors published regression equations with refe ence to the size of the probands Lung volumes could l measured in all 11 probands However the time relat flows were sufficiently measurable in 7 probands onl who cooperated adequately Blood gases were analyse before spirometry, using samples obtained from finge pricks The pulmonologist was not familiar with the prev ous history of the probands

RESULTS

Table 2 shows the results of the pulmonar function tests, giving absolute values in ml an percentage of predicted values (%) for heigh of the probands None of the lung volume measured in the 11 probands showed an statistically significant deviation from stand ards for height, and there was a significant cor relation between the test results and the age of the probands The only finding being abnormal in 6 of 7 patients where it was measur able was the expiratory flow which showed a marked decrease in the effort-independent portion with a slight increase in the total air way resistance

Table 3 lists the duration of ventilation, the highest oxygen concentration used for at least 24 hrs, the indication for the onset of the ventilator therapy, gestational ages (GA) and birthweights (BW) of the probands in connection with the ^{MEF}VC 50%/TLC, which should exceed the value of 1 There was no correlation between the severity of the pathological lung function tests and the time interval the infants spent on the ventilator, the ambient oxygen concentrations, or any of the other ventilator-settings, nor with the basic disorders justifying the starting of ventilator therapy and the gestational ages of the probands Furthermore, the interval elapsing between artificial ventilation and age of the

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Submitted Dec 10, 1978
Accepted June 11, 1979

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Table 4 *Results of blood gas analysis (peripheral samples) at pulmonary function testing*

Proband	pH	P_{O_2} (kPa)	P_{CO_2} (kPa)
1	7 38	7 45	4 00
2	7 45	8 26	3 47
3	7 34	9 60	3 33
4	7 36	10 13	4 00
5	–	–	–
6	7 41	10 66	4 27
7	7 44	10 26	4 13
8	–	–	–
9	7 37	9 46	5 87
10	7 39	0 6	4 93
11	7 45	9 60	3 73

peak pressures up to 34 0 hPa, had a GA of 30 weeks, a BW of 1200 g and developed stage 2 bronchopulmonary dysplasia (BPD) as seen on the chest X-ray At lung function testing he only had a moderately impaired MEFVC 50%/TLC of 0 8 Because of individual variation, such as maturity, it seems unlikely that a simple relationship exists between minimum duration of ventilation at a given oxygen concentration and the occurrence of BPD Because of the small number of probands available for this study we are not able to show any correlation between GA, BW, basic disorder leading to artificial ventilation, bacterial contamination and the lung function test results

Watts et al (13) measured pulmonary function in two groups of infants suffering from IRDS with and without BPD They showed an increase in arterial P_{CO_2} and increased arterial–alveolar CO_2 gradients suggesting mismatching of ventilation and perfusion They also had maldistribution of ventilation, decreased tidal volumes and higher respiratory rates being more prominent in the group with BPD Coates et al (2) found lower flow rates in air in prematurely born children with or without IRDS The authors suggest that premature birth may lead to increased resistance in the large airways and that IRDS or artificial ventilation may result in an increase in resistance in the small airways They conclude that any damage to the young respiratory bronchioli could impair the growth and further development of these terminal airways and thus explain their findings and the increased airway resistance seen by Stocks & Godfrey (9) during the first year of life in ventilated survivors of IRDS

It has been well documented in animal experiments and human pathology that artificial ventilation and oxygen administration may lead to changes in the airways ranging from severe BPD to minor histological changes (6, 8, 12) There is still considerable discussion as to whether mechanical trauma, lack of physiological humidity and temperature, high oxygen concentration, or bacterial contamination plays the leading role in the destruction of terminal airways (7, 10, 11) The findings of Taghizadeh & Reynolds (11) showed a significant correlation between high peak pressure (34 0 hPa) and lung lesions We noticed that after prolonged artificial ventilation or after ventilation with high inspiratory peak pressures the weaning from the respirator was sometimes extremely difficult and most commonly complicated by atelectasis due to mucoid impaction, needing reintubation in some cases and vigorous chest physiotherapy This could well be explained by the lesions and/or metaplasia of the epithelium of the terminal lung units (see also 10)

The results of this study tally closely with the findings of the authors cited above suggesting that the immature and vulnerable lungs are damaged primarily in the smallest air ways during artificial ventilation The damage may be the reason for the obstructively impaired expiratory pattern seen in 6 of our probands This, in turn, could facilitate lower respiratory tract infections such as severe bronchiolitis reported by other authors (1, 4) None of our probands, however, showed any subjective signs of illness

Still further studies are necessary to determine whether or not this condition persists in adult life

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Acta Pædiatr Scand 69: 165–172, 1980

THE EFFECTS OF PHARMACOLOGICAL TREATMENT ON PULMONARY FUNCTION IN CHILDREN WITH EXERCISE-INDUCED ASTHMA

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ABSTRACT [illegible]
Paediatrics, [illegible]
effects of ph[illegible]
asthma Ac[illegible]
investigated in eleven boys and eight girls (8–13 years) with a history of exercise-induced asthma (EIA), [illegible] subjective signs of EIA and a decrease in $FEV_{1.0}$ exceeding 10% after an [illegible]
ergometer and [illegible]
and five min a[illegible]
patients with [illegible]
combination of a β_2-stimulating drug and a xanthine derivative for three weeks did not significantly improve TP data. In 13 children (six from the above group) who were already on continuous treatment as above, *addition of disodium cromoglycate (DSCG) inhalations for 3–4* weeks improved the response to acute administration of a β-receptor stimulatory aerosol but did not influence EIA Seven of the children continued their DSCG treatment for one year. Minor improvement *of EIA provoked by cycling but not by treadmill was seen after this.* The ventilatory effort in relation to working intensity was lowered. No significant differences were found between treadmill running and cycling in provoking asthma.

KEY WORDS β_2-stimulating drugs, bicycle ergometry, children, DSCG, spirometry data, treadmill test, xanthine derivatives

Physical exercise frequently produces acute airway obstruction in both adults and children with asthma (15, 18) The term exercise-induced asthma (EIA) is usually applied to this condition and there are several recent reviews of the subject (2, 4, 9, 14, 23, 27) The mode of exercise has been considered to be of importance, thus running has been claimed to induce airway obstruction more than cycling or swimming (1, 2, 13) An exercise duration of 6–8 min has been reported to be optimally asthmagenic (2) Drugs, such as adrenergic β-receptor stimulating agents in aerosol, atropine and disodium cromoglycate (DSCG) given immediately before exercise protect against EIA (1, 3, 6, 8, 11, 28) On the other hand, placebos have been shown to have a significant effect on EIA (16) which suggests a multiple etiology of the condition and/or a variable lability of the airways in response to exercise

In spite of the effect of acute prophylactic treatment on EIA we believe it is important to study the influence of intensified continuous treatment on EIA in children mainly for two reasons. many children do not foresee physical activity and their activity periods are often spread out during the day We have therefore followed patients with EIA longitudinally and compared the effect of a standardized exercise on airway resistance when the children had no treatment, continuous treatment with a combination of a β_2-stimulating and a xanthine

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Table 1 *Patient data*[a]

Group[b]	No (boys)	Mean age (range) (years)	Possible heredity	Mean asthma duration (range) (years)	Positive allergy tests	Hyposensitization during trial
A	12 (7)	10 1 (8 2–13 3)	6	6 6 (2–12)	11	6
B	13[c] (8)	11 2 (8 4–13 8)	11	6 6 (1–12)	12	9

[a]

[b]

[c] Including six children (4 boys) from group A

drug and DSCG treatment, respectively Bicycle ergometer exercise has also been compared with treadmill exercise at different stages of the study in order to determine whether running provoked asthma more easily than cycling

MATERIAL AND METHODS

Eleven boys and eight girls between 8 and 13 years of age with a history of EIA were selected from a larger study of asthmatic children who were studied with complete spirometry and during exercise on bicycle and treadmill (Fig 1) The selection criterion for EIA was a 10% (2 19) or more reduction of $FEV_{1.0}$ at one or five min after exercise compared to pre exercise values Patient data are given in Table 1 The children were not allowed to take any medicine two hours before the investigations and had to be free of acute asthmatic symptoms on the day of the tests which in most cases were performed around 9 a m No studies were performed during the pollen season The tests shown in Fig 1 were completed within one week

Spirometry Total lung capacity (TLC 1 BTPS) and its subdivisions (vital capacity [VC] functional residual capacity [FRC] and residual volume [RV]) were determined by the helium dilution method using a closed spirometer system Ventilatory capacity was measured with a modified Bernstein spirometer The measurements included forced vital capacity (FEVC) forced expiratory volume in one second ($FEV_{1.0}$ 1 BTPS) the ratio $FEV_{1.0}$/FEVC=FEV% and maximum voluntary ventilation at free respiratory rate and at 40 breaths per min (MVV_{free} MVV_{40} 1 BTPS × min^{-1}) After inhalation of a β receptor stimulatory drug FEVC and its subdivisions and MVV_{40} were remeasured

Exercise tests were done both on an electrodynamically braked bicycle ergometer (Siemens Elema Sweden) and on a treadmill (Collins USA) in randomized order On the bicycle the patient pedalled in sitting position two subsequent loads corresponding to 1 5 and 2 0 watts per kg body weight each load lasting six min In two cases a third load 2 5 watts per kg was added The speed of the treadmill (in horizontal position) was adjusted so that the patients were running and their heart rate approximated 170 beats/min after six min or when performed as the second exercise test the final heart rate was the same as on the preceding bicycle test An ECG was recorded continuously during all exercise tests The lungs were auscultated before during and after exercise Wheezing coughing rhonchi or any other untoward effects were noted.

Ventilation was measured during exercise by collection of expirated gas in Douglas bags and the respiratory rate was determined Only the data obtained at the end of the exercise tests are presented Immediately before each exercise test and after its completion the $FEV_{1.0}$ and FEV% were measured again Conventional statistical methods were used Comparisons are based on paired *t* tests and corrections for changes in body size were made

PROCEDURE

Twelve of the children in the study did not receive continuous treatment at the time of the initial test (Group A) In accordance with the test plan described in Fig 2 they were placed on continuous treatment (three times daily) with the oral adrenergic β receptor stimulating drug salbutamol (Ventoline®) or terbutaline (Bricanyl®) together with a xanthine derivative usually proxiphylline (Theon®) in standard dosages After 3–4 weeks of this treatment the test procedure was repeated This is illus

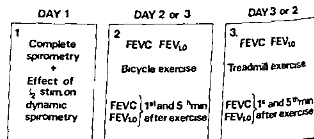


Fig 1 Investigation procedure (For explanation see text) FEVC=forced expiratory vital capacity $FEV_{1.0}$=forced expiratory volume in 1 sec

Table [illegible] ... before and after acute inhalation of a β_2-receptor stimulatory drug

Mean [illegible]
respec[illegible]
Meth[illegible]
after 3 [illegible]

	FEVC, l		FEV$_{1.0}$ l		MVV$_{40}$, l×min^{-1}		FEV%	
Group	*0*	$\Delta\beta$	*0*	$\Delta\beta$	*0*	$\Delta\beta$	*0*	$\Delta\beta$
A_I	2 22	0 22*	1 68	0 13	51	3 3	70	0 6
A_{II}	2 39	0 15*	1 80	0 12	54	3 5*	73	2 0
B_I	2 42	0 09	1 78	0 08*	53	5 0*	70	2 6
B_{II}	2 65	0 09**	2 04	0 18**	58	7 2***	72	5 9**
B_{III}	2 85	0 16	2 23	0 19	68	3 7	74	6 1*

trated in Fig 2 as A_I (the initial test) and A_{II} (the second test), respectively Seven children were already on continuous sympathomimetic treatment at the time of the initial test

These children, together with six of the children in group A, who continued to have EIA after the 3–4 week treatment period on sympathomimetics, were given DSCG (Lomudal®) inhalation 20 mg four times daily This combined group of children represent group B_I in Fig 2 After 3–4 weeks of DSCG treatment these 13 children were tested again (B_{II}) After one year of treatment with DSCG in most cases combined with continuous sympathomimetic treatment, seven of the initial 13 children were tested once more (B_{III}) The other six children had discontinued continuous treatment for various reasons The comparison between B_{II} and B_{III} is based on the seven children taking part at both occasions

RESULTS

The mean initial spirometric data of both groups are illustrated in Fig 3 The results of "static spirometry" show that both groups (A_I and B_I) had significantly increased RV, FRC and FRC/TLC compared to normals (5, 12) which indicates hyperinflation of the lungs. The results of "dynamic spirometry" reveal that both groups had decreased FEV$_{1.0}$ and FEV% as well as MVV$_{40}$ and MVV$_{free}$ compared to normal children (5, 12) The findings are consistent with obstructive lung function disturbance. There were no intergroup differences in these spirometric data or in the spirometric effects provoked by β-receptor

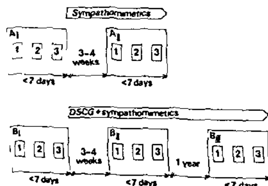


Fig 2 Investigation protocol before and after drug therapy (For explanation, see text) DSCG=disodium chromoglycole

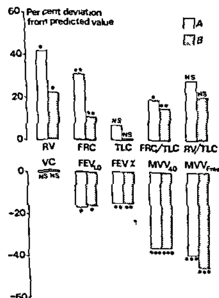


Fig 3 The initial spirometric data of groups A and B The data of Bjure and Engstrom et al (5, 12) served as normal values

Table 1. *Patient data*[a]

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[a] All patients were within ±2 S D as to body length and weight in relation to age according to Swedish standards
[b] A=continuous sympathomimetic treatment introduced B=continuous DSCG treatment introduced in a group already receiving continuous sympathomimetic treatment
[c] Including six children (4 boys) from group A

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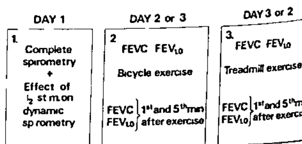


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Table 2 Spirometric data before and after acute inhalation of a β_2-receptor stimulatory drug

	FEVC l		$FEV_{1.0}$ l		MVV_{40} l×min⁻¹		FEV%	
Group	0	Δβ	0	Δβ	0	Δβ	0	Δβ
A	2 22	0 22*	1 68	0 13	51	3 3	70	0 6
A_{II}	2 39	0 15*	1 80	0 12	54	3 5*	73	2 0
B	2 42	0 09	1 78	0 08*	53	5 0*	70	2 6
B_I	2 65	0 09**	2 04	0 18**	58	7 2***	72	5 9**
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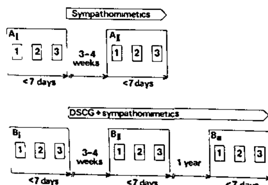


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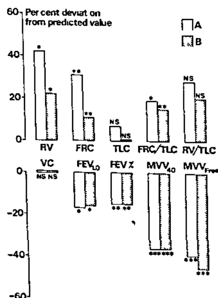


Fig 3 The initial spirometric data of groups A and B The data of Bjure and Engstrom et al (5 12) served as normal values

Table 3. *Mean values of FEV$_{1.0}$ l, BTPS and FEV% before (0) and one (1) and five (5) min after bicycle and treadmill exercise*

Absolute values are given for FEV%, while the post exercise values of FEV$_{1.0}$ represent the reductions in l BTPS from the control data. Asterisks indicate the level of significance (see Methods) in comparison with control value (0)

	Treadmill						Bicycle					
	FEV$_{1.0}$			FEV%			FEV$_{1.0}$			FEV%		
	0	1	5	0	1	5	0	1	5	0	1	5
A_I	1.76	−0.32**	−0.35**	72	70	64**	1.64	−0.16	−0.23	67	68	65
A_{II}	1.87	−0.35**	−0.39*	76	75	74	1.80	−0.20**	−0.32**	76	74	67**
B_I	1.76	−0.40*	−0.50*	74	74	66*	1.72	−0.33	−0.49*	76	73	64**
B_{II}	1.88	−0.23*	−0.42**	75	73	67**	1.90	−0.33***	−0.56***	74	71*	60**
B_{III}	2.22•	−0.26*	−0.44*	78	78	70**	2.27•	−0.19*	−0.26*	60	73	74

• Nominal value. The effect of growth is considered in the comparison between B_{II} and B_{III}

stimulation or exercise, on this initial investigation. Moreover, there was no correlation between the magnitude of the deviation from normal in these data and the duration of asthma or the age of the patient.

Treatment with sympathomimetics (Group A)

After treatment with sympathomimetics there was an increase in MVV_{free} by 14% ($p<0.05$) and shortened expiration as reflected by the FEV% (Fig. 4). Acute administration of the β_2-receptor stimulant aerosol caused a similar increase in FEVC before as well as after treatment (Table 2). The increase in MVV_{40} caused by the aerosol was not significant before but became so after treatment. The respiratory rate at rest was slightly lower after treatment than before, 18 and 20 breaths × min^{-1}, respectively ($p<0.05$). The heart rate at rest, on the other hand, was higher after treatment than before, 90 and 80 beats × min^{-1}, respectively ($p<0.01$), probably because of a direct effect of the sympathomimetic treatment. Exercise on a bicycle reduced FEVC significantly before as well as after treatment. The decrease of FEV$_{1.0}$ was more pronounced after treatment than before, hence expiration was prolonged (Table 3). Exercise on the treadmill significantly lowered FEVC, FEV$_{1.0}$ and FEV% before treatment. After treatment FEV$_{1.0}$ was still significantly decreased by exercise while FEV% was almost unchanged (Table 3).

Treatment with DSCG (Group B)

After 3–4 weeks or one year of continuous treatment 4 times daily with DSCG, no significant change occurred in the basal spirometric data (Fig. 5). However, a slight increase was noted in FEV% and MVV from the initial test B_I to the test after one year, B_{III}. Acute administration of the β receptor

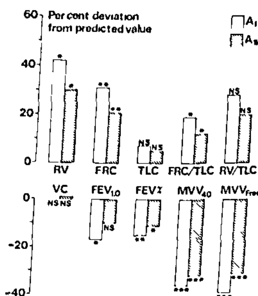


Fig. 4. Effects of sympathomimetic therapy on lung volumes and ventilatory capacity. A_I=before. A_{II}=three to four weeks after the start of drug therapy.

Table 4 *Effects of treadmill (T) and bicycle exercise (B) on heart rate and ventilation, expressed as fraction of highest spirometric measured maximal voluntary ventilation (MVV) and on tidal* ... *(VC)*

Group	Final heart rate beats × min⁻¹			Ventilation (% of MVV⁻¹)			Tidal volume (% of VC)			Respiratory rate per min		
	T		*B*	*T*		*B*	*T*		*B*	*T*		*B*
A_I	165	NS	166	63	**	51	39	NS	37	39	**	34
A_{II}	165	NS	171	55	NS	51	42	NS	46	37	*	32
B_I	169	NS	174	73	*	67	42	*	45	41	*	36
B_{II}	161*	NS	165*	66	NS	57	41	NS	43	38	NS	34
B_{III}	163	NS	167	56	NS	52	44	NS	42	36	NS	32

stimulant aerosol caused a more pronounced increase in FEV% and MVV during DSCG treatment (B_{II} and B_{III}) than before treatment (B_I Table 2)

Exercise on the bicycle ergometer significantly decreased FEVC and FEV% before, after 3–4 weeks, but not after one year of DSCG treatment (Table 3) Excercise on the treadmill decreased FEVC and $FEV_{1.0}$ before treatment as well as after DSCG treatment for 3–4 weeks and one year (Table 3) The final heart rate during exercise was lower after than before DSCG treatment following both treadmill and bicycle exercise (Table 4)

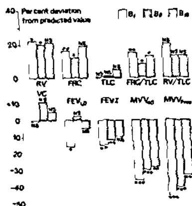


Fig 5 Effects of combined sympathomimetic and DSCG therapy on lung volumes and ventilatory capacity. B_I=before B_{II}=three to four weeks after B_{III}=one year after the start of drug therapy

Treadmill versus bicycle exercise—comparison between the respiratory effects

There were no consistent differences between the effects on the forced expiratory volume data which could be due to the type of ergometry used In fact, intraindividual comparisons of the forced spirograms revealed a probable significant difference ($p<0.05$) on only two occasions Thus, after sympathomimetic treatment (A_{II}), treadmill exercise provoked a more marked reduction of FEVC and $FEV_{1.0}$ on the first min after exercise (Table 3) On the other hand, bicycle exercise induced a more marked prolongation of expiration expressed as FEV% on the first min after exercise after prolonged DSCG treatment (B_{III}, Table 3) Comparison of the effects of treadmill work with bicycle work on heart rate and ventilation is seen in Table 4 The work intensity, expressed as final heart rate, was the same in both types of exercise during the same period of investigation although the depth and rate of breathing on some occasions attained higher values during treadmill exercise However, this higher ventilatory load was not associated with more marked signs of bronchoconstriction on the forced spirograms after the exercise

DISCUSSION

Use of a variety of exercise tests, as free running, running on a treadmill or cycling on a

Table 3 *Mean values of FEV₁ ₀ l, BTPS and FEV % before (0) and one (1) and five (5) min afte bicycle and treadmill exercise*

Absolute values are given for FEV%, while the post exercise values of FEV₁ ₀ represent the reductions in l BTPS fro the control data Asterisks indicate the level of significance (see Methods) in comparison with control value (0)

	Treadmill						Bicycle					
	$FEV_{1\,0}$			FEV%			$FEV_{1\,0}$			FEV%		
	0	1	5	0	1	5	0	1	5	0	1	5
A_I	1 76	−0 32**	−0 35**	72	70	64**	1 64	−0 16	−0 23	67	68	65
A_{II}	1 87	−0 35**	−0 39*	76	75	74	1 80	−0 20**	−0 32**	76	74	67**
B_I	1 76	−0 40*	−0 50*	74	74	66*	1 72	−0 33	−0 49*	76	73	64**
B_{II}	1 88	−0 23*	−0 42**	75	73	67**	1 90	−0 33***	−0 58***	74	71*	60**
B_{III}	2 22ᵃ	−0 26*	−0 44*	78	78	70**	2 27ᵃ	−0 19*	−0 26*	80	73	74

ᵃ Nominal value The effect of growth is considered in the comparison between B_{II} and B_{III}

stimulation or exercise, on this initial investigation Moreover, there was no correlation between the magnitude of the deviation from normal in these data and the duration of asthma or the age of the patient

Treatment with sympathomimetics (Group A)

After treatment with sympathomimetics there was an increase in MVV_{free} by 14% ($p<0$ 05) and shortened expiration as reflected by the FEV% (Fig 4) Acute administration of the β_2-receptor stimulant aerosol caused a similar increase in FEVC before as well as after treatment (Table 2) The increase in MVV_{40} caused by the aerosol was not significant before but became so after treatment The respiratory rate at rest was slightly lower after treatment than before, 18 and 20 breaths × min⁻¹, respectively ($p<0$ 05) The heart rate at rest, on the other hand, was higher after treatment than before, 90 and 80 beats × min⁻¹, respectively ($p<0$ 01), probably because of a direct effect of the sympathomimetic treatment Exercise on a bicycle reduced FEVC significantly before as well as after treatment The decrease of $FEV_{1\,0}$ was more pronounced after treatment than before, hence expiration was prolonged (Table 3) Exercise on the treadmill significantly lowered FEVC, $FEV_{1\,0}$ and FEV% before treatment After treatment $FEV_{1\,0}$ was still significantly decreased by exercise while FEV% was almost unchanged (Table 3)

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After 3–4 weeks or one year of continuous treatment 4 times daily with DSCG, no significant change occurred in the basal spirometric data (Fig 5) However, a slight increase was noted in FEV% and MVV from the initial test B_I to the test after one year B_{III} Acute administration of the β receptor

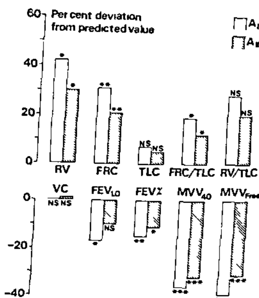


Fig 4 Effects of sympathomimetic therapy on lung volumes and ventilatory capacity, A_I—before A_{II}=three to four weeks after the start of drug therapy

may vary in different patients (8) As mentioned in the introduction, sympathomimetics as well as DSCG in aerosol form have a preventive effect when given immediately before exercise However, few reports have appeared about the effects of continuous long term treatment on proneness to EIA During a long term trial with DSCG it was shown that premedication with DSCG prevented EIA better in children with a satisfactory clinical response to the drug in other respects than in those who failed to respond Without premedication there was no difference in the EIA response, i e, bronchial lability was not affected (26) An improved response to DSCG premedication but no effect without premedication has been reported after three weeks of DSCG treatment (11) Oral premedication with sympathomimetic drugs seems rather inefficient in preventing EIA in contrast to aerosol administration of the same drugs (3, 24) The mechanism for this finding has not been determined One possible explanation is a lower drug concentration at the receptor site after oral administration (3) Our findings with long-term medication are in agreement with these studies In this connection it is interesting to note that long term steroid treatment does not seem to influence proneness to EIA significantly (20, 21) which suggests that there is no simple relationship between EIA and the severity of other asthma manifestations

In the present study we found no significant differences between treadmill running and cycling in provoking asthma This might be due to the loads that were used Treadmill running and cycling at loads of 1 5 and 2 0 watts/kg body weight have been reported to be equally asthmagenic if a particular threshold of exertion is exceeded Based on laboratory observations such a threshold has been placed at a heart rate of 150 or 160 per min (9) Hence the final heart rates in the present study of about 170 per min would be adequately provocative

In conclusion continuous peroral treatment with a combination of β receptor stimulant drugs and a xanthine derivative for 3–4 weeks did not significantly improve EIA according to the spirometric data Addition of daily DSCG inhalations for 3–4 weeks did not change this finding, but improved the response to acute administration of a β-receptor stimulant aerosol Prolongation of this treatment to one year improved EIA provoked by cycling but not by treadmill However, identical work was then performed with a lower ventilatory effort, and five children out of seven claimed that they had less EIA.

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bicycle ergometer during different exercise periods and of different loads on cardiorespiratory function have led to conflicting results concerning the sensitivity of the tests employed to detect EIA (1, 7, 10, 22) In addition to differences in methodology, variations in the pathophysiological pattern of the obstructive lung disease also need to be taken into consideration Large and small airways may be differently affected even in the same individual at different times Hence lung function tests of two regions of the bronchial tree may give apparently varying results It seems that mild and moderate forms of EIA are primarily due to obstruction of the large airways (9) and may be diagnosed by decreases of specific airway conductance or airway resistance measurements FEVC, $FEV_{1.0}$ and the FEV% reflect changes in large and/or small airways Small airway obstruction, on the other hand, can be detected by tests of, for example, maximal midexpiratory flow rate The majority of investigators of EIA, however, have relied upon measurements of $FEV_{1.0}$ and peak expiratory flow rate from forced expirograms and Wright's peak flow meter, respectively As the latter instrument cannot be calibrated, the $FEV_{1.0}$ determinations with a Bernstein spirometer are preferable in physiological investigations The interval between the two exercise tests within one investigation period, i e , less than one week, should give the least coefficient of variation according to studies of the variability of EIA (25)

The children in the present study were selected because of subjective complaints suggestive of exercise induced asthma which could be verified by at least a ten per cent reduction of $FEV_{1.0}$ after exercise tests on a treadmill and/or bicycle ergometer No other criteria for selection were used except for the exclusion of children under the age of eight years who usually cannot participate adequately in these tests This selection made the groups heterogeneous both as to asthma duration and type and degree of allergy Fourteen of the nineteen children were being treate or had been treated with allergen injectio and/or hyposensitization which may have i fluenced the results of the long term DSC treatment

In the majority of the patients the individu spirometric data at the beginning of the stud were within the 95% confidence limits (healthy children (5, 12) However, the grou means of those without (A_1) and with conti uous previous treatment (B_1) differed signif cantly from the predicted values This woul suggest the presence of hyperinflation an bronchoconstriction with little or no effect b acute administration of the β_2 receptor stim lant drugs Both groups had similar spir metric characteristics and responded similar to exercise Short term, continuous treatme for 3–4 weeks, with a β_2 receptor stimulatin drug in combination with a xanthine derivati did not essentially change the response t acute administration of the β_2 receptor stim lating aerosol Addition of DSCG treatmen for 3–4 weeks somewhat improved this re sponse which might reflect a beneficial in fluence of DSCG on, for example, mucosa oedema and secretion

The response to exercise was not signifi cantly influenced by the short term treatmen either with sympathomimetics alone or i combination with DSCG Even after one yea of DSCG treatment the results are conflicting an improvement of the response to bicycl exercise was seen while the response to tread mill exercise seemed to be essentially un changed Five of the seven children who too DSCG for one year, however, claimed to hav less EIA This subjective improvement ma have a physiological explanation in that the performed the same work with less ventilator effort expressed as a lower fraction of thei MVV and at a lower respiratory rate

The exact mechanism for EIA remains obscure in spite of extensive studies Recently, direct pharmacological evidence for a multi factorial etiology has been obtained (3, 17, 28) and the relative importance of each factor

Acta Pædiatr Scand 69 173–176, 1980

DEEP ACTINOMYCOSIS IN CHILDHOOD

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ABSTRACT. Stanley, T. V. (Royal Hospital for Sick Children, Glasgow, U K). Deep actinomycosis in childhood Acta Paediatr Scand, 69, 173, 1980.—Visceral actinomycosis is extremely rare in childhood Two cases of visceral actinomycosis in children are described, and the importance of accurate mycological diagnosis and early treatment emphasized. The outcome in one case would very probably have been fatal had the diagnosis not been considered promptly, and in the other the child would have received ineffective chemotherapy. A new observation is the presence of a defective immune response during the acute infection which returns to normal during convalescence.

KEY WORDS Visceral actinomycosis, childhood, immune deficiency

Actinomycosis is a very rare disease of childhood (1), and visceral actinomycosis even more so, only three cases appearing to have been published in the last decade Two cases of visceral actinomycosis presenting at this hospital within three months of each other are reported to underline the importance of early diagnosis and treatment in this dangerous but eminently treatable disease A new observation in this disorder, namely a transient immune deficiency, is described

CASE REPORTS

Case 1 S E This 10-year-old son of healthy parents presented with a five month history of weight loss, lethargy and pallor For three months he had complained of anorexia nocturnal rigors and malaise There was no relevant past medical or family history and there had been no recent trauma On examination he was noted to be pale and anxious He was apyrexial very thin his height 138 cm (between 50th and 75th centiles) his weight 25 7 kg (at the 10th centile) Pulse rate was 132 beats per min Examination of respiratory cardiovascular, abdominal and central nervous systems was normal Examination of urine was normal Investigations showed a haemoglobin of 80 g/l total white cell count of 25×10^9/l (25 000/mm³) (70% neutrophils) E S R was 90 mm in the first hour Urea and electrolytes calcium and liver function tests were normal with the exception of a total plasma protein of 98 g/l Gammaglobulin concentration was 55 3 g/l All three major immunoglobulin classes were raised

Chest X ray showed a small effusion at the left base and partial collapse of the left lower lower lobe Connective tissue disease screen was negative, as was a viral screen Bone marrow examination showed an increase in plasma cells and reticuloendothelial iron block Mantoux was negative Intravenous urography showed normal renal outlines, the left kidney being rotated about its transverse axis Ultrasound of abdomen demonstrated a spleen enlarged in its transverse diameter

In the week following admission the child s condition deteriorated and he lost a further 1 5 kg A diagnostic pleural tap was carried out with difficulty, producing a small quantity of whitish yellow material Microscopy revealed polymorphs and lymphocytes but no organisms were seen, and routine culture was negative

A barium meal showed the stomach to be displaced anteriorly and to the right by a soft tissue mass (Fig 1) At laparotomy an abscess cavity was found superolateral to the spleen, and a further abscess cavity in the right posterolateral subphrenic space Three small hard nodules were found in the liver Bilateral drains were inserted

Subsequent to laparotomy, Actinomyces spp were isolated from both pleural and abscess fluid and he was started on high dose penicillin therapy parenterally Ten days postoperatively S E developed radiological signs of a pericardial effusion confirmed by ultrasonography, but this subsided with conservative therapy He made an uneventful recovery receiving a total of four months therapy with penicillin

Immunological studies ten days after operation showed depressed cellular immunity, with negative delayed hypersensitivity skin tests to Candida, Mumps and Streptococcal antigens, although lymphocyte responses to mitogen, polymorph function tests, and complement screen were normal Repeat skin tests ten weeks later were strongly positive

Case 2, M McB This 11 year-old fourth son of healthy family presented with a four month history of intermittent sharp left sided chest pains lasting 2–3 days at a time Two weeks before admission he had developed a moist

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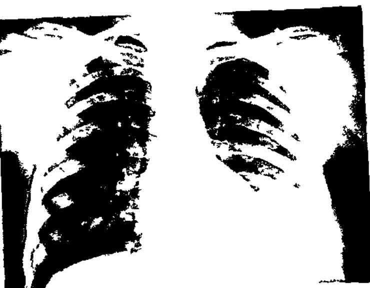


Fig 3 Case 2 Chest X ray showing left pleural effusion

Actinomycosis is reported to be a mixed infection the anaerobe *Actinobacillus actinomycetemcomitans* being the commonest accompanying organism (5, 6) and failure to respond to therapy may be due to the resistance of this organism No other organisms were isolated in these two cases

Actinomyces israelii is sensitive to all the commonly used antibiotics penicillin and tetracyclines being the most effective, prolonged therapy appears more important than high dosage (4) and surgical drainage of abscess cavities may be necessary for eradication of the organism

Although three cases of superficial actinomycosis in children have recently been reported (2), there appears to have been only three reports of visceral actinomycosis in childhood in the last decade (3, 8, 10) and we were surprised to discover two cases in such a short period The possibility that this condition is becoming more common in Western Europe should be considered

We learnt an important lesson from each of these cases Case 1 was severely ill and would undoubtedly have died had the diagnosis not been reached In neither case were characteristic 'sulphur granules' seen on naked eye examination of specimens

Case 2 underlines the importance of bacteriological confirmation, if possible prior to institution of therapy, even in cases of presumed osteomyelitis Actinomycotic pericardial effusion has been well described in the past (9) and usually responds to antibiotic therapy alone

The phenomenon of temporary anergy returning to normal during convalescence is known to occur in a wide range of infections (11) the commonest of which are probably measles and infectious mononucleosis, however, it does not appear to have been previously described in actinomycosis It is not clear if the immune depression in these patients was primary, leading to a commensal organism becoming a pathogen, or secondary to the infection itself The return to normal of immune function with recovery supports the latter view It would be valuable to study immune function in cases of more localised actinomycosis, and in older patients

In Case 2 negative delayed hypersensitivity skin tests were accompanied by demonstrably abnormal *in vitro* lymphocyte function tests This was not seen in Case 1, which might suggest the mechanism of immune depression was different in this child, such as defective macrophage function, this was not investi-



Fig 1 Case 1 Barium meal showing displacement of stomach to the right by a soft tissue mass

cough and three days prior to admission had noticed a very tender swelling on the left anterior chest wall There had been no weight loss and he was otherwise well There was no history of trauma

Past medical and family history was unremarkable

On examination he appeared generally well Height and weight were both at the 25th centile for age He was apyrexial There was a large red tender non fluctuant swelling anterolaterally on the left side of the chest extending into the axilla adherent to both skin and ribs (Fig 2) There was bilateral axillary and cervical lymphadenopathy There were clinical signs of a large left sided pleural effusion Heart rate was 130 beats per min

Examination of cardiovascular abdominal and central nervous systems was otherwise normal

A chest X ray confirmed the presence of a large left sided pleural effusion and soft tissue swelling over the 5th to 7th ribs (Fig 3)

A clinical diagnosis of empyema secondary to osteomyelitis of ribs was made and orthopaedic advice sought It was suggested the best guess choice of therapy was a combination of fucidin and erythromycin However it was decided to attempt aspiration of the mass which had become fluctuant after 24 hours and thick yellowish pus aspirated revealed large quantities of Actinomyces spp Other investigations showed him to have a haemoglobin of 98 g/l total white cell count 15 6×10⁹/l (15600/mm³

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exception of a total plasma protein of 60 g/

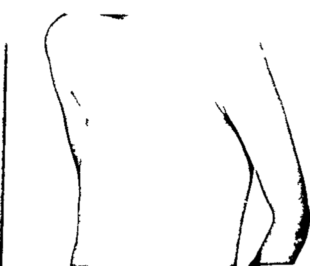


Fig 2 Case 2 showing swelling over the anterior che wall on the left

globulin of 44 g/l A viral screen was negative Repe chest X ray three days after admission showed evidenc of subperiosteal reaction over the affected ribs

Delayed sensitivity skin testing with Candida Mump and Streptococcal antigens again produced no respons and on this occasion mitogen stimulation of lymphocyte using phytohaemagglutinin and pokeweed induced a poo response although rosette techniques showed norm quantities of B and T lymphocytes

When skin tests were repeated after an interval of 1 weeks on treatment they induced a strongly positive re sponse and on this occasion lymphocyte stimulat on pro duced a normal response

He made an uncomplicated recovery with high dos penicillin therapy

DISCUSSION

Virtually all cases of actinomycosis in man ar caused by *Actinomyces israelii* which can onl exist in parasitic form (6)

It is an anaerobic gram positive organism with a ray structure having a peripheral zone of gram negative clubs Pus from lesions ca be shown to contain sulphur granules i shaken with water *Actinomyces israelii* is thought to be a common commensal in the gastrointestinal tract (7) in particular in the presence of diseased tissue such as caries or appendicitis and to be introduced into the site of infection by trauma However, both children had healthy teeth in Case 1 the appendix was noted to be normal and neither case had a history of trauma

Acta Pædiatr Scand 69 177–182, 1980

DTP AND DTP-INACTIVATED POLIO VACCINES COMPARISON OF ADVERSE REACTIONS AND IGG, IGM and IGA ANTIBODY RESPONSES TO DTP

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ABSTRACT. Ruuskanen, O, Viljanen, M K, Salmi, T. T, Lehtonen, O-P., Kouvalainen, K and Peltonen, T. (Departments of Paediatrics and Medical Microbiology, University of Turku, and Department of Paediatrics, University of Oulu, Finland). DTP and DTP inactivated polio vaccines comparison of adverse reactions and IgG, IgM and IgA antibody responses to DTP. Acta Paediatr Scand, 69:177, 1980.—Adverse reactions and anti DTP antibody responses were compared between DTP- or DTP inactivated polio-vaccinated children The material consisted of 380 children whose adverse reactions were registered by detailed questionnaires given to the parents IgG-, IgM- and IgA-anti-DTP antibodies of 42 children were quantified using enzyme-linked immunosorbent assay (ELISA) Fever, restlessness and local reactions were the most frequent adverse reactions observed DTP polio vaccine induced significantly more restlessness than DTP. This was the only significant difference in adverse reactions between the vaccines. An enhancement of IgG-anti DTP antibody responses at the age of 6 months was observed in the DTP-polio group. The enhancement was transient in antitoxin responses but still present in pertussis antibodies at 8 months of age. Very low and mostly undetectable levels of IgM- and IgA-anti DTP antibodies were observed in both groups.

KEY WORDS. IgG, IgM and IgA antibodies, DTP, DTP-inactivated polio, ELISA

Immunization with a combination of vaccine antigens has proven to be a safe and effective way to reduce the cost of vaccination programs (9) The basic combination is diphtheria tetanus and pertussis (DTP) vaccine, which is known to induce good antibody responses and a few adverse reactions (8) DTP vaccine has been combined further with many viral antigens, e g , inactivated polio vaccine (1) or live attenuated measles vaccine

In Finland two different immunization schedules are used The only difference between these programs concerns the vaccination at the age of 5 months when DTP vaccine or DTP combined with inactivated polio vaccine can be used Immunization with the combination has been shown to enhance the antibody response against polio (3, 5) and slightly also against diphtheria and tetanus (3). There is a general impression among the public health workers that DTP-polio vaccine would induce more adverse reactions than the third DTP vaccination at the corresponding age However, no firm data about a comparison of the adverse reactions is available. In this work we have studied the adverse reactions and antibody responses against DTP of these two vaccination programs An ELISA technique was applied for the quantification of IgG, IgM and IgA antibodies against DTP. No earlier data are available on the class-specific antibody response against DTP.

SUBJECTS AND METHODS

The material consisted of 380 children whose adverse reactions and antibody responses were monitored as shown in Table 1 The results concerning the polio vaccinations will be published separately

gated. However, *in vitro* lymphocyte function tests are widely thought to be relatively crude criteria of *in vivo* lymphocyte function (12) and it may be more advanced techniques will shed more light on this observation

ACKNOWLEDGEMENT

I would like to thank Professor White and his staff at the Western Infirmary, Glasgow for doing the immunological studies, and Laboratory Staff at the Royal Hospital for Sick Children

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Submitted May 15, 1979
Accepted Aug 31, 1979

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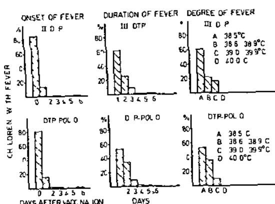


Fig. 1. Comparison of onset, duration and degree of fever in children vaccinated with DTP or DTP inactivated polio vaccine.

as standards throughout the study. When expressed as units/ml the concentration of the reference serum for anti-tetanus antibodies was 36 U/ml. This figure was arrived at when the reference serum was compared in several dilutions to a commercial antiserum (Tetgam Inject, Behringwerke AG, Frankfurt am Main, Germany). No corresponding comparison was available for anti-diphtheria and antipertussis antibodies.

Statistics

The Chi square test was used to compare the incidence of adverse reactions in the different groups.

Student's *t* test was applied for statistical analysis of log transformed data about antibody concentrations.

RESULTS

Adverse reactions

Questionnaires were returned by 76–90% of the parents. Fever reaction, restlessness and local reactions were the most common adverse effects. Fever reaction (≥37.5°C per rectum) was reported after the DTP vaccinations in 46–54% of the children. The III DTP-I polio vaccination at the age of 5 months induced fever reaction in 60% of the children, while the corresponding figure after the III DTP vaccination was 54%. The frequency of fever reaction after the IV DTP-III polio at the age of 24 months was 35%. When compared to the III DTP-I polio vaccination the difference is significant ($p<0.001$). There were no differences in the degree and quality of fever reactions after the different vaccinations with DTP vaccine. Furthermore, the fever reactions after the III DTP and the III DTP-I polio vaccination were of a similar nature (Fig. 1). The reactions started in the majority of the children on the day of the vaccination and lasted 1–2 days. Fever reaction exceeding 39.0°C was reported in 10% after the III DTP vaccination and in 6% after the III DTP-I polio vaccination. Fever reaction did not exceed 40°C in any of the children. The III DTP-I polio vaccination induced significantly more restlessness than the III DTP vaccination, 48% and 36%, respectively ($p<0.05$). Local reactions were found in 60% of the children after the IV DTP-III polio vaccination and in 30–32% after the other vaccinations. The frequency of the other adverse reactions was small and did not exceed the control values, i.e., the existence of same kind of symptoms at the time of the vaccination among the unvaccinated siblings or playmates. Fever drugs were given to 14–26% of the children. The difference between the III DTP and the III DTP-I polio vaccination in this respect was significant ($p<0.05$).

IgG anti DTP antibodies (Fig. 2)

After three DTP vaccinations at the age of 6 months all of the children had concentrations of IgG anti DTP antibodies easily measurable by the ELISA method. A significant decrease of antibody concentrations occurred between the ages of 6 and 8 months. Diphtheria and tetanus antibodies reached their highest level at the age of 25 months, one month after the booster vaccination. Concerning pertussis antibodies the corresponding booster effect was not so distinct and the mean concentrations did not exceed those at the age of 6 months. No significant correlation existed between the antibody responses against the individual antigens. A good responder against one antigen could have a low response against the other and *vice versa*.

The concentrations of IgG anti DTP antibodies at the age of 6 months were higher in the group vaccinated with DTP polio vaccine than in the group vaccinated with DTP vac-

Table 1. *The vaccination program and serum collections*

Age (months)	190 children	190 children	42 children
3	I DTP	I DTP	
4	II DTP	II DTP	
5	III DTP	III DTP-I polio	
6	I polio	II polio	Serum sample
7	II polio	–	
8	–	–	Serum sample
24	IV DTP-III polio	IV DTP-III polio	
25	–	–	Serum sample

Vaccines and immunizing schedules

Commercially licensed DTP and DTP-inactivated polio vaccines from the same lot were used in all of the immunizations except that at the age of 24 months DTP vaccine (The Central Public Health Laboratory, Helsinki, Finland) contained 50 Lf/ml diphtheria toxoid, 10 Lf/ml tetanus toxoid and 10^9 pertussis bacteria per milliliter DTP-polio vaccine (Orion Diagnostica, Helsinki, Finland) was prepared by adding inactivated polio vaccine (Vaccine antipoliomyelitique, R I T, Genval, Belgium) to DTP vaccine of the same lot in equal volumes Toxoids were adsorbed with aluminium phosphate and mixed with pertussis bacteria DTP vaccine was given in a volume of 0 5 ml and DTP-polio vaccine in 1 0 ml

The immunizing schedule used in the study is presented in Table 1 The children received DTP vaccine at the ages of 3 and 4 months At 5 months of age half of the children were vaccinated with DTP vaccine and the other half received DTP-polio vaccination All of the children received DTP polio vaccination at the age of 24 months The serum samples for antibody quantifications were taken at the ages of 6, 8 and 25 months (Table 1)

Registration of adverse reactions

Data concerning the adverse reactions after the vaccinations were collected by using questionnaires given to the

2. The reactions after the IV DTP III polio vaccination at 24 months of age were recorded from a separate group of 200 children not included in the original material However, the serum samples at that age were taken from the same children as the previous samples

Immunoassay for anti DTP antibodies

The quantification of IgG, IgM and IgA antibodies against DTP was carried out by enzyme-linked immunosorbent assay (ELISA) earlier used in our laboratory for antibodies against *Yersinia* bacteria (4) and tetanus toxoid (13)

The antigens were the same as those produced for vaccines, but not supplemented with aluminium phosphate (Orion Diagnostica) The purity of diphtheria and tetanus toxoids was 1875 and 2340 Lf/mg protein N respectively Formalin killed *Bordetella pertussis* bacteria were used as antigen in ELISA (Per-vaccin, batch 1/76, concentration 10^{11} bact /ml, Orion Diagnostica)

The tests were carried out in disposable polystyrene

diphtheria toxoid (4 7 Lf/ml), tetanus toxoid (3 5 Lf/ml) or *Bordetella pertussis* organisms (57×10^8 bact /ml) was added to the cuvettes The cuvette blocks were incubated at +37°C for 3 hrs and washed three times with

... 500 and for the IgM and IgA tests ... The ELISA procedure used is described in detail elsewhere (13)

the reference serum The same reference sera were used

Table 2. *Precentage of adverse reactions to the different vaccinations*

Reaction *N* (%)[a]	I DTP 343 (90)	II DTP 330 (87)	III DTP 143 (79)	III DTP-I polio 144 (76)	IV DTP-III polio 153 (77)[c]
Fever (≥37 5°C)	46	52	54	60	35
Restlessness	36	40	36	48	34
Local reaction	30	30	31	32	66
Vomiting	9	10	4	9	5
Diarrhoea	3	3	3	7	7
Contact with nurse/physician	3	3	5	1	1
Fever drugs given	14	19	18	26	14
Controls[b]	6	6	0	7	2

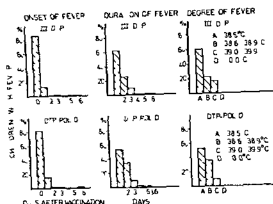


Fig 1 Comparison of onset duration and degree of fever in children vaccinated with DTP or DTP inactivated polio vaccine

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IgG anti DTP antibodies (Fig 2)

After three DTP vaccinations at the age of 6 months all of the children had concentrations of IgG anti DTP antibodies easily measurable by the ELISA method A significant decrease of antibody concentrations occurred between the ages of 6 and 8 months Diphtheria and tetanus antibodies reached their highest level at the age of 25 months, one month after the booster vaccination Concerning pertussis antibodies the corresponding booster effect was not so distinct and the mean concentrations did not exceed those at the age of 6 months No significant correlation existed between the antibody responses against the individual antigens A good responder against one antigen could have a low response against the other and *vice versa*

The concentrations of IgG anti DTP antibodies at the age of 6 months were higher in the group vaccinated with DTP polio vaccine than in the group vaccinated with DTP vac

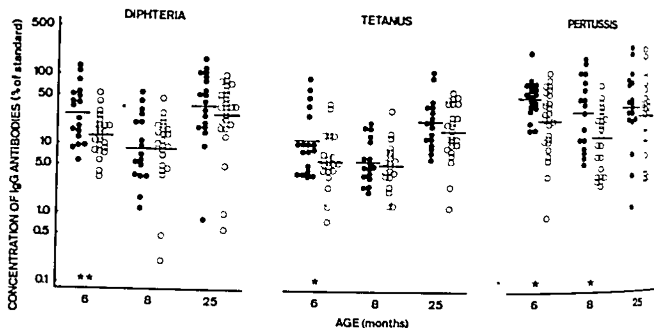


Fig. 2. IgG antibodies against diphtheria, tetanus and pertussis in children vaccinated with DTP inactivated polio at the age of 5 months (closed circles) and in children immunized with DTP vaccine at the same age (open circles). Geometric means indicated by horizontal bars. Asterisks indicate the statistical significance of the differences between the groups. * $p<0.05$, ** $p<0.01$.

cine. The difference was most prominent in diphtheria antibodies ($p<0.01$) but reached statistical significance also in tetanus and pertussis antibodies ($p<0.05$). Concerning pertussis antibodies the difference was significant two months later ($p<0.05$) and even at the age of 25 months antibodies against all three antigens were on a higher level in the DTP-polio group than in the DTP group, but the differences were not statistically significant.

IgM- and IgA-anti-DTP antibodies

In the vast majority of the children the concentration of IgM and IgA antibodies was below the detection limit of the method used. Only in a very few serum samples could these antibody classes be observed and even then their concentrations were only slightly above the lowest measurable concentrations.

DISCUSSION

Few definite data are available about the adverse reactions after the DTP and DTP-inactivated polio vaccinations. In the present study using detailed questionnaires we found that fever, restlessness and local reactions were the most frequent adverse effects of these vaccinations. Fever reaction was recorded in half of the vaccinees. It must be emphasized, however, that the parents were asked to watch the children carefully and measure the body temperature whether or not they suspected fever reaction. Clinically significant fever reaction (≥39°C) was found in less than 10% of the vaccinees. No difference was observed in this respect between the successive DTP vaccinations. When the adverse reactions after the III DTP vaccination and III DTP-I polio vaccination given at the same age were compared, the only difference was that DTP-polio vaccine induced significantly more restlessness. This possibly explains the higher frequency of fever drug medication after the DTP-polio vaccination. The high incidence (66%) of local reactions after the IV DTP III polio vaccination at the age of 2 years is probably due to the hypersensitivity state of the individuals with high levels of anti-DTP antibodies (12). Our experience is that the questionnaire method is reliable in the recording of adverse reactions after vaccina

tions This is supported by our earlier studies When using the same type of questionnaires we found that live measles vaccine induced a pattern of adverse reactions clearly different from that of the DTP polio vaccination e g fever reaction started much later on an average 9 days after the vaccination (10)

Both immunization schedules induced good IgG antibody levels against DTP in all the children Even the lowest observed concentration of tetanus antibodies (0 9%) was almost three hundred times higher than the concentration commonly considered to be protective against the disease (0 01 U/ml−0 0036%) (6) A similar estimation of the protective effect of IgG antibody levels against diphtheria and pertussis cannot be carried out due to the lack of human reference sera with a known concentration of antibodies Their concentration was 10–100 times higher than before the onset of the vaccination program at the age of 3 months (14) Diphtheria vaccination has also proved to be clinically highly efficacious since the last patient with diphtheria was found in Finland in 1955 By contrast the efficacy of pertussis vaccination is a subject of debate Although in this work we observed IgG antibodies against pertussis in concentrations methodologically comparable to the antitoxoid antibodies the clinical protection rate of the vaccination has been shown to be only 60–70% (2 11)

Immunization with the combination of inactivated poliovaccine with DTP enhanced IgG antibody responses to DTP at the age of 6 months The phenomenon was transient in the antitoxoid responses and its significance is probably limited A significant enhancing effect could be observed in IgG antibodies against pertussis still at the age of 8 months This may have some benefical effect on the efficacy of the pertussis vaccination Our results relating to the enhancement of anti DTP response by inactivated polio vaccine confirm the earlier findings (3)

One reason for the low or undetectable levels of IgM and IgA anti DTP antibodies may be the fact that the samples were collected one month after the vaccinations and these antibodies have disappeared However, IgA antibodies play an important role in the local protection of mucous membranes against infective agents such as *Bordetella pertussis* IgA has probably not such an important function in the protection against bacterial toxins Thus the lack of or at least the very transient existence of IgA antibodies after DTP vaccinations may be one of the factors decreasing the prophylactic effect of the vaccination against pertussis The immune response after natural whooping cough differs from that after vaccination containing marked elevations of both IgM and IgA antibody levels This probably enables us to make the serological diagnosis of the disease from a single serum sample (14)

In conclusion it can be stated that the combination of inactivated polio vaccine with DTP vaccine does not increase the incidence of adverse reactions and enhances IgG antibody response against DTP

ACKNOWLEDGEMENTS

This work was supported by a grant from the National Board of Health Finland We wish to thank Mrs Maija Salonen and Mrs Marjo Ingman for their skillful technical assistance

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Submitted April 20 1979
Accepted Sept 7, 1979

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Acta Pædiatr Scand 69 183–187, 1980

ZINC AND IMMUNE FUNCTION IN DOWN S SYNDROME

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Paediatr Scand, 69 183, 1980.—Low levels of zinc in se... They also had an immune deficiency found in 12 patients with Down s syndrome (DS) characterized by depressed neutrophil chemotaxis skin hypersensitivity and lymphocyte ... The patients received zinc sulphate, corresponding to 135 mg

improved in 10 of 11 patients after zinc therapy ... increased susceptibility to infection in DS

KEY WORDS Down s syndrome, zinc, immune deficiency, neutrophil chemotaxis

Patients with Down s syndrome (DS) suffer from frequent infections and have an increased mortality in infectious diseases compared to a normal population (30) Several laboratory studies have demonstrated abnormalities of cell mediated and humoral immune capacity and of phagocyte function (3 4, 7, 24 27)

Low serum zinc levels have been reported in children with DS (20, 23) Zinc is involved in various cellular processes such as membrane phenomena, messenger RNA metabolism many enzyme reactions (6 16) and may be associated with immunological abnormalities e g in acrodermatitis enteropathica (AE) (26) and malnutrition (9, 10)

In a previous study of DS patients was found depressed PHA induced lymphocyte stimulation and low skin hypersensitivity after DNCB sensitization (3) Neutrophil chemotactic responsiveness was also impaired but other phagocyte functions were found normal In the present study we report that oral zinc therapy to children with DS improves their serum zinc levels and enhances neutrophil chemotaxis as well as T cell function

PATIENTS AND METHODS

Patients Twelve patients with DS 5 girls and 7 boys 8 to 22 years of age (mean 16 years) were studied Informed consent was obtained from their parents and the study was approved by the Ethical Committe at the University of Umeå

Zinc therapy Zinc was administered orally as zinc sulphate (Solvezink® Tika AB Lund Sweden) for 2 months The children had a body weight of 30 kg or more and received 600 mg of zinc sulphate daily corresponding to 135 mg Zn^{++} divided in three doses Venous blood was obtained through cubital vein puncture before and after 2 months of treatment The blood samples were drawn in the morning and the patients had fasted overnight

Determinations of trace elements Serum blood clot and hair concentrations of zinc and copper were determined by atomic absorption spectrophotometry (Varian AA-6DB) as previously described (11, 17)

Chemotaxis Neutrophil chemotaxis was measured by the leading front technique in modified Boyden chambers as described by Wilkinson (28) using Millipore filters with 3 µ pore size and 5% zymosanactivated (Zymosan Sigma Co St Louis Mo) pooled human serum as cytotaxin

DNCB sensitization and test Sensitization was performed by the application of 25 µl of a 2% solution (w/v of DNCB (1-chloro-2 4-dinitrobenzene) in aceton to a skin

Table 1. *Zinc and copper (mean ± S E) in serum, blood clots and hair from patients with Down's syndrome (DS) (n=12) and healthy controls (n=83)*

	Zinc			Copper		
	Serum (μmoles/l)	Clots (μmoles/kg)	*Hair* (mmoles/kg)	Serum (μmoles/l)	Clots (μmoles/kg)	Hair (mmoles/kg)
DS before zinc therapy	11 7±1 0[a]	620 7±42 2[b]	2 53±0 63	18 9±1 2	47 4±7 2[b]	0 22±0 14
DS after zinc therapy	24 3±5 7	659 0±76 0	2 59±0 46	16 4±1 4[c]	47 9±38 8	0 21±0 09
Controls	15 4±2 5	441 0±56 2	2 22±0 75	19 5±0 05	39 6±5 1	0 24±0 17

[a] Differs significantly from controls and DS patients after therapy ($p<0$ 001)
[b] Differs significantly from controls ($p<0$ 001)
[c] Differs significantly ($p<0$ 05)

area of 3 cm² The skin was covered with an impermeable plastic film for 72 hours About 4 weeks later the patients were tested epicutaneously with graded amounts of DNCB A patch test response in a normal subject provided *erythema* papules, vesicles and infiltration 72 hours after challenge with 5 or 10 μg of DNCB Erythema alone after application of 20 μg of DNCB was considered as a *weak response* Patch tests with more than 20 μg of DNCB applied to the circular test area (diameter 10 mm) regularly gave toxic reactions Rechallenge with DNCB was performed about 6 months later, i e immediately after the two month period of zinc therapy

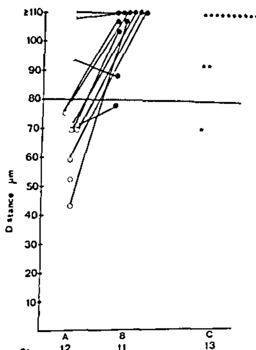


Fig 1 Chemotaxis by netrophils from patients with DS before (*A*) and after oral zinc (*B*) and from healthy controls (*C*) Values obtained from the same patients are joined by a line The dotted line denotes the lower level of neutrophil chemotaxis considered as normal when healthy adults and children are studied in our laboratory

Lymphocyte stimulation Lymphocytes were obtained from defibrinated venous blood after separation on Lymphoprep® (Nyegaard Co , Oslo, Norway) The cells were washed and suspended in RPMI Hepes (Gibco Madison Wisc) supplemented with antibiotics and 15% autologous serum and incubated in Microtest II tissue culture plates (Falcon Plastics, Los Angeles Calif) with PHA (Leucoagglutinin, Pharmacia AB, Uppsala Sweden) for 3 days Culture conditions and measurement of DNA synthesis have been previously described (13 25)

Immunoglobulins The immunoglobulins A, G and M were determined by the radial immunodiffusion technique using Partigen plates (Behringswerke AG Marburg W Germany)

Statistics The differences between group means for different variables were tested using Student s *t* test for unpaired observations The test was modified if the variances were significantly different ($p<0$ 01 *F* test) Wilcoxon s signed rank test for paired observations was used for comparison of the skin test results

RESULTS

The concentration of zinc and copper in serum, blood clots and hair are shown in Table 1 Before treatment zinc levels were lower in serum ($p<0$ 001) and higher in clots ($p<0$ 001) in the DS patients compared to the controls Zinc levels in hair did not differ significantly between DS patients and controls The copper concentrations were normal in serum and hair but increased in the clots in the DS patients ($p<0$ 001)

Zinc therapy considerably increased the levels of zinc in serum ($p<0$ 001), while the concentrations of zinc in clots and hair were not changed significantly Concentrations of copper in serum were decreased compared to

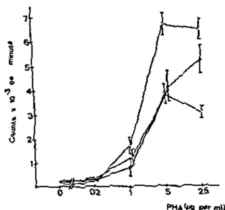


Fig 2 The response to PHA of lymphocytes from patients with DS before (○) and after (●) initiation of therapy and from healthy persons (□) The lymphocytes were incubated for 3 days in the presence of various concentrations of PHA The incorporation of [¹⁴C] thymidine into DNA was measured

the controls while the concentrations in clots and hair were not significantly influenced by zinc treatment

In nine of twelve patients decreased neutrophil chemotactic responsiveness to zymosan activated serum was found prior to therapy (Fig 1) After zinc therapy decreased chemotactic responsiveness was present in only one patient

Initially skin reactivity to DNCB was weak or absent in 9 of the 12 patients tested When the tests were repeated 6 months later the contact allergic response to DNCB was increased in 10 patients ($p<0\ 05$) One of the two patients who did not show an increased skin reactivity already had a maximum response initially

In the pre treatment period lymphocytes from the DS patients showed a decreased response to various doses of PHA compared to lymphocytes from the controls (Fig 2) After two months of zinc therapy the lymphocyte response to 25 μg PHA per ml was improved ($p<0\ 05$) whereas responses to lower doses were unchanged

Zinc therapy did not influence levels of immunoglobulin in sera of the patients (Table 2)

DISCUSSION

All the DS patients had low serum zinc concentrations, and the values did not overlap with those obtained from the controls This study supports previous studies (20, 23) The DS patients were all normal with respect to serum albumin This indicates that the low serum zinc levels were not caused by a decreased concentration of this major zinc binding protein of plasma When the blood samples were obtained, none of the patients suffered from an infection depressing plasma zinc concentration (2) Increased zinc concentration was found in blood clots from the patients A previous study has demonstrated increased zinc concentration in erythrocytes from a small number of DS patients (20) The findings indicate defective zinc metabolism in DS blood cells The presence of true zinc deficiency in DS was supported by the clinical effect of therapy since the response to therapy with zinc is probably one of the most reliable indices for making a diagnosis of zinc deficiency in man (21) During treatment, serum zinc concentrations increased to values over those in the controls The decrease in serum copper concentration after zinc therapy has been observed previously (14) No patients showed any signs of adverse effects of the zinc therapy However, in a continuing study we are giving the patients only 400 mg of zinc sulphate daily

Neutrophils of the DS patients had decreased chemotactic responsiveness to cytotaxins generated in zymosan activated serum This confirms previous studies (3, 15) The

Table 2 *Immunoglobulin levels (g/l) of DS patients before and after zinc therapy*

	IgA	IgE*	IgG	IgM
Before	3 1±1 8	33±61	13 6±2 9	0 44±0 15
After	2 8±1 2	19±17	13 6±3 3	0 49±1 7
Normal range	0 5±3 0	10±400	5±15	0 4±1 7

* U/ml

chemotactic responsiveness of the neutrophils was reduced by about 30%, but returned to normal in most patients after zinc therapy The mechanism of leukocyte locomotion is very complex involving both membrane and intracellular phenomena (29) Zinc concentration and phagocyte activity are associated and both inhibitory and stimulatory effects of zinc have been reported on various functions (5) Interference with divalent cations, microtubuli or metabolic enzyme systems are some of the proposed zinc dependent regulatory mechanisms involved (18)

The DNCB skin reactivity was also improved after zinc therapy, suggesting that the expression of the delayed hypersensitivity was enhanced Since repeated skin testing implies a challenge of the immune system, a booster effect cannot be excluded There was, however, a 6-month period between the tests and this interval reduces the booster effect Our results also support the findings in children with protein energy malnutrition and low plasma zinc levels who showed increased delayed skin hypersensitivity after topical treatment of the test site with zinc ointment (10) Recently animal studies have conclusively demonstrated that zinc deficiency may depress immune function independent of mal nutrition (19)

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This work was supported by a grant from the Swec Medical Research Council (B77 19X 04990)

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Submitted May 8 1979
Accepted Aug 29 1979

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Acta Pædiatr Scand 69 189–192, 1980

AUTOANTIBODIES TO TAMM-HORSFALL PROTEIN IN PATIENTS WITH CYSTIC FIBROSIS

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From the Department of Paediatrics and the Department of Immunology Institute of Medical Microbiology, University of Goteborg and the Department of Paediatrics, University Hospital Umeå Sweden

ABSTRACT. Fasth, A and Kollberg, H. (Department of Paediatrics and Department of Immunology, Institute of Medical Microbiology, University of Goteborg, and Department of Paediatrics, University Hospital, Umeå Sweden) *Autoantibodies to Tamm-Horsfall protein in patients with cys* [illegible] patients, 17 boys and 18 [illegible] Tamm-Horsfall protein [illegible] but not IgM antibodies to Tamm Horsfall protein were found. There was a considerable overlap between the values in the disease group and the control group. The highest values were found among the patients with liver involvement. The patients with marked lung abnormalities as well as those with positive bacterial culture of sputum had normal antibody levels.

KEY WORDS Autoantibodies, Tamm-Horsfall protein, cystic fibrosis, liver involvement

Although cystic fibrosis (CF) is the most common lethal to semilethal inherited disease of children and young adults in Caucasian populations, its underlying biochemical defect is still unknown Earlier observations pointed to a possible abnormality in lysosomes (1, 3, 14, 15, 18) Recent studies have shown that in the majority of CF fibroblast cultures the activity of alkaline phosphatase can be induced with the Tamm-Horsfall (TH) glycoprotein (10, 11, 12) and that in these cultures many lysosomal enzymes leak out of the cells into the culture medium (12) This finding reopens the question if TH protein may be involved in the pathogenesis of CF

MATERIAL AND METHODS

Patients

Serum was taken from 35 patients with CF, of whom 17 were boys and 18 girls having an age range of 2–16 (mean 9) years The diagnosis was based on at least one of the major clinical findings meconium ileus, pancreatic insufficiency and/or chronic pulmonary obstructive disease and confirmed by elevated sweat electrolytes (7) None of the patients had or had had any known urinary tract infection, as judged by regular routine urine examinations Two boys were judged to have liver involvement due to an enlarged liver with the border 3 cm below the right costal margin Chest x ray and/or lung spirometry were performed on all the patients Sputum from each patient was cultured for bacterial growth

The serum samples were obtained as a part of regular examinations and were analysed for antibodies to TH protein for bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and creatinine

Reference material for the TH antibody levels was the same as earlier used (5) The control population included 70 children with an age of 1–20 years

TH protein was prepared from human urine by precipitation with 0 58 M NaCl, as described earlier (5)

The enzyme linked immunosorbent assay (ELISA) of Engvall & Perlman (4) was used for determinations of TH antibodies with modifications, as described earlier (5) The antibody levels were expressed as percentages of the mean of the reference material

RESULTS

The results of determination of TH antibodies are shown in Table 1 and Fig 1 Many patients had elevated levels of IgG and IgA antibodies in comparison with a reference population of corresponding age. The difference between the means of the reference population and the patients was highly significant ($p<0\ 001$) for

suggests an immunization against the TH glycoprotein or some cross-reacting structure

The absence of any signs of urinary tract infection, either at the time of testing or in the past, as judged by routine urinary examination and normal serum creatinine values makes urinary tract disease less likely as the cause (5)

Up to 25% of CF patients are found to have some liver involvement at autopsy, but liver involvement with clinical problems is much more rare (5–10%) and occurs mainly in the newborn period with prolonged neonatal jaundice (17) and/or in adolescence with biliary cirrhosis, sometimes with portal hypertension (16) The great majority of CF children, however had no clinical findings of liver involvement even if a slight involvement could be revealed by laboratory tests and liver biopsy

Patients with liver diseases have increased levels of autoantibodies to the TH protein (6), and cross reactions between TH and liver structures have been demonstrated (2) The highest antibody levels to TH were found among the children with signs of liver involvement Thus there is a strong possibility that liver abnormalities contribute to the increased TH antibody levels Most CF patients get pulmonary infections, most often caused by *Staphylococcus aureus* or *Pseudomonas aeruginosa* Our continuing studies indicate a partial identity between the TH glycoprotein and the R core structure of *Escherichia coli* lipopolysaccharide (8)

It was also reported recently that CF patients have increased numbers and amounts of precipitating antibodies against bacteria of the normal faecal flora (9) The reason for this is unknown but the authors speculate on the possibility of increased absorption of antigens over mucosal surfaces Although one might expect cross reactions, we could not find any correlation between the levels of TH antibodies and bacterial findings

There was a considerable overlap between the values found in the CF patient material and in the control population This overlap indicates inhomogeneity and a reasonable explantion seems to be that only the patients with liver involvement get elevated TH antibody levels The question as to whether the TH glycoprotein plays any pathogenetic role in CF, as proposed by Hosli & Vogt (12), is still unsolved

ACKNOWLEDGEMENTS

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technical assistance

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10 Hosli, P, Erickson R P & Vogt E Prospects for prenatal diagnosis of cystic fibrosis Induction of biochemical abnormalities in fibroblasts from patients

Table 1. *Levels of Tamm-Horsfall protein antibodies in serum from children with cystic fibrosis*
The antibody levels are expressed as a percentage of the mean of the reference material

Clinical group	Number	Tamm Horsfall antibodies (Mean±standard deviation) IgG	IgA
Total material	35	154± 90 $p<0.001$	198±107 $p<0.001$
Boys	17	166±107 $p<0.02$	237±121 $p<0.01$
Girls	18	127± 72 n s	154± 78 $p<0.01$
Liver involvement	7	271± 78 $p<0.001$	302±127 $p<0.01$
Severely abnormal spirometry and/or chest x ray, no liver involvement	10	81± 26 n s	147± 91 n s
Positive sputum culture	13	118± 59 n s	165± 97 n s
Reference material	70	100± 30	100± 40

The mean values were compared with those of the reference group using the Student s *t* test, n s = not significant

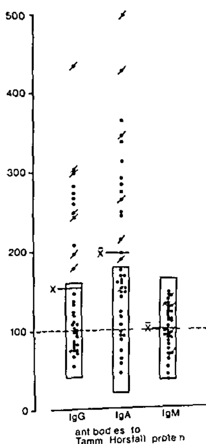


Fig 1 Levels of antibodies against Tamm Horsfall glycoprotein in sera from CF patients expressed as percent of the mean of the reference material The boxed areas represent the mean levels ± 2 S D for the reference material The mean value for the CF material in each antibody class is indicated with a bar
✗=seven children with liver involvement (5 children with increased serum aspartate transaminase and 2 boys with enlarged liver)

the IgG and IgA, but not for the IgM antibodies to the TH protein

All patients had normal values of bilirubin creatinine, alanine transaminases, and alkaline phosphatase Another 5, apart from the 2 with liver enlargement, had aspartate aminotransferase levels at +2 S D or more Of the 2 patients with liver enlargement, one had abnormal spirometry, and of those with elevated transaminases one had both abnormal chest x-ray with bronchectasias and decreased spirometric values In the sputum of 13 patients *Staphylococcus aureus* was found and in 4 of those *Pseudomonas aeruginosa* was also cultured Ten patients had moderate to severe impairment of their spirometry and/or marked abnormalities at their chest x-ray When clinical and laboratory parameters were compared with the levels of IgG and IgA antibodies to TH the highest amounts were found among the 7 patients (6 boys and one girl) with liver involvement In patients with either severe lung damage or positive bacterial culture the TH antibody levels were not significantly elevated No difference in the antibody levels was found between the sexes

DISCUSSION

The increased levels of IgG and IgA antibodies to TH protein in serum from patients with CF

suggests an immunization against the TH glycoprotein or some cross-reacting structure.

The absence of any signs of urinary tract infection, either at the time of testing or in the past, as judged by routine urinary examination and normal serum creatinine values makes urinary tract disease less likely as the cause (5)

Up to 25% of CF patients are found to have some liver involvement at autopsy, but liver involvement with clinical problems is much more rare (5–10%) and occurs mainly in the newborn period with prolonged neonatal jaundice (17) and/or in adolescence with biliary cirrhosis, sometimes with portal hypertension (16) The great majority of CF children, however, had no clinical findings of liver involvement even if a slight involvement could be revealed by laboratory tests and liver biopsy

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ACKNOWLEDGEMENTS

This work was given financial support by the "Forenade Liv" Mutual Life Group Insurance Company, Stockholm, Sweden, and by the Swedish Medical Research Council (No 215) Thanks are due to Ingrid Sutherland for skilful technical assistance

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Submitted Sept 3, 1979
Accepted Oct 17, 1979

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Acta Pædiatr Scand 69 193–199, 1980

LYMPHOCYTE SUBPOPULATIONS IN PATIENTS WITH VARIOUS IMMUNODEFICIENCIES

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ABSTRACT Gmelig Meyling, F , Dollekamp, I , Zegers, B J M , Ballieux R E and Stoop, J W (University Children s Hospital Het Wilhelmina Kinderziekenhuis ' and Department of Clinical Immunology, University Hospital, Utrecht, The Netherlands) Lymphocyte subpopulations in patients with various immunodeficiencies Acta Paediatr Scand, 69 193, 1980.—We have studied patients with various immunodeficiencies for the occurrence of l [illegible] bearing [illegible] different surface markers the affinity to sheep erythrocytes (E [illegible] cel [illegible] the [illegible] Ig [illegible] fo [illegible] sIg bearing or E_m-binding B lymphocytes [illegible] cells and 3 out of 4 also had low proportions of IgM-FcR bearing (T) cells A child with partial DiGeorge syndrome did not have a grossly abnormal marker pattern although there appeared to be a shift in the T/B cell ratio in favour of the B cells In a patient with selective cellular immunodeficiency associated with a purine nucleoside phosphorylase deficiency, who was followed during reconstitution therapy with plasma and red cells, a positive change in the marker pattern was seen Similar observations were made in a child with combined immunodeficiency during treatment The findings are being discussed in the light of the current knowledge of the functions and ontogeny of lymphocyte subsets

KEY WORDS Immunodeficiency lymphocyte surface markers, lymphocyte subpopulations

The use in clinical immunology of methods to physically enumerate lymphocyte sub populations has contributed considerably to a better understanding of many immunological abnormalities The arsenal of these cell sur face marker tests is constantly being extended, particularly with tests for markers which are thought to be characteristic for ontogenetic and functional subsets of the two major lym phocyte subpopulations, T and B cells Two examples of such possible subset markers are the affinity of some B cells for mouse erythrocytes (13) and the receptor for IgM which is expressed predominantly on the majority of T cells (20) Naturally, the application of such novel marker tests requires the constant accu mulation of relevant normal values This has been the subject of a previous paper in which six marker tests (among which the above duo) were performed on blood lymphocyte samples of immunologically normal neonates and young children (7) The main purpose of that study was to contribute to the body of normal values which is still relatively modest for the youngest age groups The present paper deals with the application of the six marker tests and of the information acquired on their nor mal values, to the study of some patients with immunodeficiencies

PATIENTS

(*a*) Patient R V developed a *selective cellular im munodeficiency* during the first months of her life based

plasma (23) Detailed clinical immunological and metabolic findings have been published (28)

(*b*) Patient C R the first child of healthy unrelated parents was admitted to our hospital at the age of two years with longstanding diarrhea associated with a *Salmonella typhimurium* infection She had a severe lymphopenia, a low percentage of E_s binding T cells a diminished response of the lymphocytes on *mitogenic and* allogeneic stimulation and no response on DNCB challenge after appropriate sensitization Serum IgM *IgA and IgD* was not detectable and the serum IgG (about 5 mg/ml) consisted predominantly of IgGl Gm(f) type λ molecules Specific antibodies against anamnestic antigens could not be detected No sIg *bearing B* lymphocytes were demonstrable in blood and bone marrow These data led to the diagnosis of *combined immunodeficiency* The patient was *treated* with thymosin (10) and thymic humoral factor (29) without clinical improvement or changes in the outcome of *in vitro* immunological tests A subsequent regimen of repeated plasma transfusions however *appeared to* be clinically successful Also an increase in the number of blood lymphocytes and in the percentage of E_s binding T cells occurred (see Results and Discussion)

(*c*) Patient A O was admitted at the age of 1 month for the evaluation of congenital heart disease She had a mild hypertelorism and *malformed* low set ears hypocalcaemia was established but the patient had no tetany No thymus shadow was seen on thorax X ray pictures The blood lymphocyte counts were normal but the percentage of E_s binding T cells was *slightly* below the age level However *in vitro* mitogenic and allogeneic lymphocyte stimulation was unimpaired and the levels of serum IgM IgG and IgA were normal The cardiovascular anomalies consisted of a patent ductus arteriosus and anomalous right subclavian artery During thoracotomy no thymus was found In a lymphnode biopsy the paracortical areas contained only few lymphocytes It was concluded that probably an *incomplete DiGeorge syndrome* existed in this patient

(*d*) Four patients with *congenital agammaglobulinaemia* were investigated An extensive report on the clinical and immunological status of these patients will be published elsewhere (30)

(*e*) Finally four patients with *ataxia teleangiectasia* were studied

METHODS

In this study tests for six lymphocyte surface markers were performed There markers were the affinity of T cells for sheep erythrocytes (E_s) the surface immunoglobulins (sIg) of B cells the affinity of B cells for mouse erythrocytes (E_m) and the receptors for the Fc part of IgM (IgM-FcR) the Fc part of IgG (IgG-FcR) and for complement (CR) Methodological details and the specificity of these tests have been described elsewhere (6 7)

IgG-FcR occurring on a T cell subset were demonstrated by a double marker technique in which the E_s were labelled with fluorescein and the ox red cells (used for the assessment of IgG-FcR) labelled with rhodamin (6) prior to their sensitization with IgG-class antibodies mixture of the green and red fluorescent indicator erythrocytes was used for this rosette test

Lymphocyte cultures for determination of mitogen-induced lymphocyte stimulation were performed as described elsewhere (9)

RESULTS AND DISCUSSION

(*a*) As is shown in Table 1, the lymphocyte surface marker pattern of patient R V was highly abnormal prior to therapy the proportion of E_s binding T lymphocytes was low and no IgM-FcR positive lymphocytes were demonstrable Although there was a severe lymphopenia, the percentage of B cells was normal

When the patient was treated with plasma and irradiated erythrocytes, the lymphocyte counts increased to about 1 000/mm³ coinci dently with the treatment This increase was however, always of a transient nature This picture of peaks and dips remains up to the present time, during this period, twenty plasma and/or red cell infusions were admin istered (not fully covered by Table 1) The percentages of E_s binding T cells showed gradual and constant increase Two addition points must be noted the fact that within month after the beginning of the treatmen IgM-FcR positive lymphocytes became de monstrable and the increase in the absolut and relative numbers of E_m binding lymph cytes which were repeatedly equal to or eve higher than the sIg bearing B lymphocytes

The low percentage of E_s binding T cells in this patient is undoubtly associated with he selective cellular immunodeficiency The absolute numbers of B lymphocytes however were found to be decreased as well Neverthe less, her immunoglobulin and antibody levels in serum were not grossly abnormal, adequate numbers of plasma cells were present in the bone marrow and the class distribution of their cytoplasmic immunoglobulins was normal (28) When blood lymphocytes were cultured for six days with the B cell activator Pokeweed mitogen, the lymphocyte yield

able 1 *Longitudinal observations in two patients with immunodeficiency*

ge (months)	Blood lymphocyte count/mm³	% of lymphocytes bearing the following markers[a]: E_s	IgM FcR	T cells bearing IgG–FcR[b]	sIg	E_m
atient R V ♀ Selective cellular immunodeficiency						
ior to reatment	204–450	20–29	<1	–[c]	13–20	1–5
c d	279	16	<1	–	14	1
|	1 316	22	4	–	17	22
| 5[c]	225	23	2	–	29	17
|	1 265	45	4	–	23	10
| 5	400	46	10	–	15	12
c	336	42	2	–	17	5
5	1 095	46	9	–	14	13
atient C R ♀ Combined immunodeficiency						
nor to reatment[f]	200–700	20–39	<0 5		<0 5	7 8
)	7 100	90	1	22	0 3	<1
|	1 600	78	1 2	16	<0 1	<0 2
|	3 000	63	0 6	–	<0 1	1 4
)	1 640	60	5 2	40	0 8	3 7
7	4 030	70	3	22	<0 1	5
ormal values & range[a])–2 years of age	6 300 {2 150–10 700}	62 {49–73}	59 {14–86}	18 {6–35}	13 {5–22}	7 {2–15}

ons

–λ ml

after culture was low but normal percentages of plasma cells containing IgM, IgG and IgA were present which indicates the presence of helper cell activity

Infusion of irradiated lymphocytes and plasma led to a change in lymphocyte marker tests in this patient Later on, it could be shown that this change was entirely due to the erythrocyte transfusions and that concurrently the metabolic state of the patient altered dramatically (33)

The rise in the proportion of E_s binding T cells in this patient can be explained on one hand by amelioration of the metabolic state of the lymphocytes but on the other hand, the treatment could also induce or allow the maturation of non-E_s binding T cell precursors into E_s-binding T cells Anyhow, the percentage of R V 's lymphocytes which reacted with a well-defined anti T cell serum (3) appeared to be considerably higher than the proportion of E_s-rosettes (28, 33), this indeed accounts for the high percentage of unidentifiable lymphocytes (E_s- and sIg negative) It should be noted that the acquisition during ontogeny of the affinity to E_s is preceeded by the expression of certain T cell specific differentiation antigens (2) The appearance of some IgM–FcR positive cells during treatment might be connected with a T lymphocytic maturation process since among thymocytes, only relatively few cells express this surface marker and then, probably only in a low density (6, 8) The IgM-binding T cell population in normal individuals has been shown to contain cells helping B cell activation *in vitro* in terms of

mitogen-induced differentiation (17) and antigen-driven antibody production (*15*, *26*) (whereas cells suppressing such activities reside in the IgG–FcR-bearing T cell subset) Apparently, the lack of IgM–FcR-bearing T cells in this patient was not allied to an absence of T helper cell function *in vitro* because Pokeweed mitogen did cause plasma cell differentiation, indicating that helper T cell function is not necessarily coupled to expression of the IgM–FcR This is also shown by the helper capacity of human thymocytes in a similar experimental system (16)

The increase during therapy of E_m-binding B cells could indicate the appearance in the circulation of relatively young B cells (*13*), either as a direct result of the enzyme therapy, or as a consequence of a certain restoration of T cell function leading to an increased proliferative capacity of lymphocytes of the B cell axis

(*b*) The results of lymphocyte surface marker tests in *patient C R* (*Table I*) prior to plasma transfusion therapy supported the diagnosis of combined immunodeficiency Her T lymphocyte percentage was low, varying from 20 up to 39% as determined on different occasions, and sIg-bearing B lymphocytes were absent This picture has been observed in seven out of eighteen similar patients studied by others (27) At one occasion, the E_m-rosette test was performed and 8% of E_m-binding cells were found IgM–FcR positive cells were not demonstrable

After the start of the plasma transfusions, a rapid increase in the absolute and relative numbers of E_s-binding T cells was noted, this was allied to an increase of her total lymphocyte count to a normal level The sIg bearing B cells remained extremely low or were even absent However, lymphocytes with affinity for E_m were regularly demonstrated IgG–FcR bearing T cells were always present during the year in which the observations were made In this patient, as in patient R V, IgM–FcR-bearing lymphocytes appeared during treatment Interestingly, on the same day when the highest percentage of IgM–FcR positive cells was scored (12%, 42 months of age) the *in vitro* mitogenic lymphocyte stimulation which was previously very low, appeared to be perfectly normal (results not shown in Table I)

The finding of E_m-binding lymphocytes in this patient in the (virtual) complete absence of normal sIg-bearing B lymphocytes is intriguing Such a proportion of sIg/E_m does not occur among normal blood lymphocytes (6) but might be present in organs containing relatively many "young" B cells and/or B cell precursors, such as the foetal liver (13) The possibility that these sIg^-/E_m^+ cells could also be early maturational stages of leukocytes other than (B) lymphocytes, not normally occurring in blood (c f the occurrence of Ia-like antigens on granulocyte precursors, Ref (31)) cannot wholly be excluded at the moment Anyhow, E_m binding cells of this patient were unable to proceed to terminal stages of B cell differentiation when cultured with Pokeweed mitogen, even in the presence of normal T lymphocytes

The coincident finding of a relatively high percentage of IgM–FcR-bearing lymphocytes and a normal mitogenic stimulation in this patient is interesting It suggests that it is particularly the IgM–FcR-positive T cells that are being brough to division by mitogens Indeed indications for this have recently been found in normals (18) On the whole, however, the proportion of IgG– and IgM–FcR- positive (T) cells did not change dramatically during therapy, although E_s-binding T cells did increase in number Such a pattern (low IgM–FcR-bearing T cells and normal IgG–FcR bearing T cells) has also been observed in other patients with combined immunodeficiency (*11*, 19)

(*c*) The outcome of lymphocyte marker tests in patient A O are shown in Table 2 The absolute numbers of sIg-positive and E_m-binding cells were normal, but their percentages were quite high which agrees with the relatively high percentage of CR-bearing lymphocytes, about half of which are B cells in

Table 2 *Observations in other patients with various immunodeficiencies*

Patient	Sex	Age	Blood lymphocyte count/mm³	% of lymphocytes bearing the following markers E_r	IgM–FcR	sIg	E_m	IgG–FcR Total	On T[a]	CR
Partial Di George syndrome										
A O[b]	♀	1 month	3 640	39	39	26	19	–[c]	33	34
Congenital agammaglobulinaemia										
R B	♂	9 years	1 500	61	61	<0 2	<0 2	23	–	14
R L	♂	7 years	2 200	62	63	<0 2	–	9	11	13
R O	♂	15 years	3 100	72	41	<0.5	<0 2	42	–	23
G V	♂	10 years	2 700	76	–	<0 5	<0 2	–	6	11
Ataxia teleangiectasia										
E B	♂	18 years	2 130	46	25	1	1	29	10	18
R E	♂	18 years	1 550	61	24	1	3	32	–	25
H L	♂	10 years	1 100	77	32	2	2	26	–	30
H S	♀	19 years	1 100	57	51	1	3	20	16	24
Normal adults[b] average & range			2 200 (1 050–3 950)	69 (53–88)	59 (45–68)	9 (3–24)	5 (1–11)	26 (12–45)	18 (6–35)	26 (10–45)

[a] Number of IgG–FcR bearing cells per 100 T cells determined by double marker test
[b] For values in normal children from 0–2 years of age see Table 1 and Ref 7
[c] Not determined

normals (6) The percentual increase in B cells was most probably related to the moderately decreased percentage of T cells This shift in the T/B cell ratio however, was less marked than may be the case in similar patients (5) In addition the proportions of IgM–FcR and IgG–FcR bearing T lymphocytes were within the normal range which has recently also been found by other workers in 2 out of 3 similar patients studied (11, 19)

(*d*) The patients with congenital agammaglobulinaemia all had normal proportions of T lymphocytes (Table 2) No sIg positive B lymphocytes could be demonstrated, which would agree with the existence in these patients all of which were males, of sex linked (Bruton type) agammaglobulinaemia (5, 24, 25, 32) This diagnosis could however, not be made with certainty in all four cases E_m binding B lymphocytes were also lacking in the patients tested which agrees with observations made elsewhere (12) CR bearing lymphocytes were quite low, although not abnormal Thus, too, is in agreement with a number of other studies (1, 12, 14, 17, 25, 32) This receptor, which is often taken as a specific marker for B lymphocytes, is demonstrable on a considerable number of sIg negative 'non-T' lymphocytes in normals (6) It is still unclear whether the cells are related to B cells in normals or in these patients (who may have B cell precursors, Ref 4 and 21)

The proportions of the IgG and IgM binding T cells were within normal limits

(*e*) Of the patients with ataxia teleangiectasia (Table 2), at least one (E B) had decreased T cells In another patient, H S , the *absolute* number of T cells was reduced The percentages of CR– and IgG–FcR positive lymphocytes (the latter both determined on total lymphocytes and on T cells) were not abnormal However, few B lymphocytes were demonstrable In three patients, IgM–FcR-bearing lymphocytes were markedly reduced Even if the presence of this receptor on some B lymphocytes is taken into account (22), it is clear that these patients have a deficit in the IgM–FcR positive subset This confirms the

outcome of a recent study by Gupta & Good (11) Some workers have found that the percentages of E_a-binding T cells are normal or slightly decreased in patients with ataxia telangiectasia (12, 25), others found clearly diminished percentages (5, 17) This might be explained by differences in the technique for E_a-rosette formation and betray a relatively low affinity of the patients' T cells for E_a

In general, normal percentages of B cells are found (5, 12, 17, 25) which disagrees with the low B cell proportions in our four patients This may be caused by the fact that in the present study, we have used an incubation at 37°C to remove cytophilic IgG from non B cells (7) The majority of the 'B cells' in the patients studied by Schiff et al (25) did, in fact, carry IgG on their surface

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Submitted Jan 8, 1979
Accepted Aug 15, 1979

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Acta Pædiatr Scand 69 201–204, 1980

THE CORD SERUM FREE AMINO ACID LEVELS IN APPROPRIATE AND SMALL FOR GESTATIONAL AGE NEWBORN INFANTS OF MOTHERS WITHOUT CLINICAL MALNUTRITION IN ABIDJAN

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ABSTRACT Reinhardt, M C and Burkhalter, J F (Department of Immunology, Institute of Child Health, London, England and Laboratoire Central, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland) The cord serum free amino acid levels in appropriate and small for gestational age newborn infants of mothers without clinical malnutrition in Abidjan Acta Paediatr Scand, 69 201, 1980.—The cord serum amino acid levels were determined in nine small for gestational age and fourteen appropriate for gestational age newborn infants of Abidjan, Ivory Coast Small for gestational age newborns had a significantly lower total amount of amino acids, but the characteristic deviation of the individual concentrations and the high glycine/valine ratio seen in experimental and clinical protein deficiency were not found

KEY WORDS Cord blood, newborn, small for gestational age, amino acids, malnutrition, developing country

Foetal growth may be impaired by a number of maternal placental, foetal and environmental factors The importance of an unknown maternal factor has been demonstrated (20) Small size for gestational age therefore has a highly multifactorial pathogenetic background and does not automatically imply pre natal nutritional deficit

Maternal diet during pregnancy has an important effect on birthweight (1, 7, 10, 18) The pattern of the free amino acids in plasma changes characteristically in kwashiorkor and in experimental protein deficiency (13) The raised glycine/valine ratio has been found to be a valuable tool in the diagnosis of postnatal subclinical protein deficiency (2, 3) and has been found to be raised in cord blood plasma in very poor socio-economic groups of Pakistan (12) and Ethiopia (8) with a grossly deficient maternal dietary intake during pregnancy

The aim of the present study was therefore to investigate whether the infants of our sample whose birthweight was below the 10th percentile of Lubchenco's intra uterine growth curves (15) showed the characteristic deviations in their blood amino acid levels which would indicate protein deficiency during pregnancy

MATERIALS AND METHODS

In a previous study (16) of 204 births in Abidjan Ivory Coast 14 small for gestational age newborn infants (TSGA) were examined In nine of these cases it was possible to analyse the amino acids from cord blood serum samples Sampling procedures and general information about the mothers and their infants have been published (16) The results were compared to those of a group of 14 randomly selected appropriate for gestational age new born infants from the same population (TAGA)

Gestational age was determined by one of the authors (M C R) using clinical criteria (6) which have been shown to be applicable to coloured newborn infants (9, 17) Blood pressure was not measured in the mothers As analyses were performed on serum samples it is not possible to compare the present results with the results of plasma analyses In our sample the mothers showed no clinical signs of important protein-calorie malnutrition

Table 1 *Anthropometric and haematological data*

	TAGA (N=14)	TSGA (N=9)	p value for Student's t test
Anthropometric data			
Weight for height of mothers (% of normal value)	100±13*	94±10	NS
Triceps skinfold thickness of mothers (% of normal value)	65±26	55±20	NS
Height of mothers (cm)	161± 6	156± 5	<0 05
Weight of newborns (kg)	3 05± 0 3	2 37± 0 1	<0 001
Gestational age (wks)	39 0± 0 2	39 1± 1 3	NS
Haematological data			
Haemoglobin newborns (g/100 ml)	15 2± 1 9	15 8± 2 0	NS
Packed cell volume newborns (%)	43 7± 5 7	46 3± 4 5	NS

* Mean ±1 S D

(16) Weight for height of the mothers had a normal distribution as compared to standards of developed countries but triceps skinfold thickness was markedly below the normal value The TSGA newborns had mothers with significantly lower height but the other anthropometric measurements of the mothers were similar in the two groups (Table 1)

Cord blood samples were centrifuged after clotting within one hour separated kept at 4°C for a maximum of six hours and then stored at −20°C until analysis Deproteinization was carried out with an excess of crystalline sulphosalicylic acid Amino acids were measured by ion exchange chromatography according to the method of Spackman et al (19) using a Biotronik 6000 LC (Munich) automatic amino acid analyser A lithium buffer system was used (4) to permit the separation of glutamine asparagine threonine and serine However glutamic and aspartic acid could not be measured because of the increased levels of their amides produced by deamination during the 6 months storage between sampling and analysis (5) Trytophan levels could not be measured by this method due to partial binding of this amino acid to albumin Furthermore cystine was not measured because of likely losses during storage and handling of the samples (19)

RESULTS AND DISCUSSION

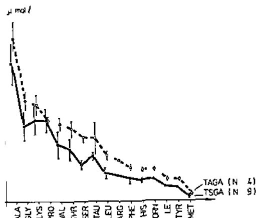
The results are shown in Table 2 and in Fig 1 The levels of almost all amino acids were significantly lower in TSGA than in TAGA infants and the TSGA group had significantly lower total amino acid level than the TAGA group (Student's t test, $p<0.001$) There was however no significantly difference in the glycine/valine ratio between the two groups The high proline, alanine and glycine and low valine concentrations observed in Kwashiorkor and in low birthweight newborns of low socio economic groups in Pakistan and Ethiopia (8) were not observed in the S newborn infants of the present sample

In the absence of maternal amino a values one can only speculate about the cau and significance of the lower overall am acid levels of cord serum in TSGA ne born infants As TSGA newborns had hig

Table 2 *Free amino acids in cord ser (µmol/l)*

	TAGA (N=14)	TSGA (N=9)	p value for Student's t test
Taurine	175± 88*	127± 27	<0 05
Threonine	202± 44	146± 52	<0 01
Serine	178± 21	103± 24	<0 001
Proline	227± 27	226± 52	NS
Glycine	281± 43	209± 57	<0 01
Alanine	453± 87	382± 83	<0 05
Valine	214± 34	157± 55	<0 01
Methionine	20± 7	12± 5	<0 01
Isoleucine	61± 16	43± 17	<0 05
Leucine	127± 23	81± 31	<0 05
Tyrosine	59± 14	32± 15	<0 001
Phenylalanine	93± 15	63± 24	<0 01
Ornithine	83± 19	58± 22	<0 01
Lysine	267± 53	226± 61	NS
Histidine	83± 23	59± 9	<0 01
Arginine	112± 23	72± 25	<0 01
Total amino acids	3 190±380	2 350±510	<0 001
Glycine/valine ratio	1 32±0 16	1 22±0 33	NS

* mean ±1 S D



? 1 Cord serum free amino acid levels of 9 at term
all for gestational age (TSGA) and 14 at term approp
te for gestat onal age (TAGA) newborn infants Mean
' S E M

acked cell volume and haemoglobin values than TAGA newborns this cannot be ex lained by haemodilution The results contra ict the assumption that short gestation should e the cause of the low birth weights of the nfants in cord blood of fullterm newborns here is an increase of methionine taurine and ysine (11 14) which was not found in these TSGA newborns

The increase of non-essential amino acid levels (glycine proline) and in particular the increased glycine/valine ratio (13 2 3 8) char acteristic of protein deficiency was not found in the present series of TSGA Our findings indicate that these TSGA may not suffer from foetal growth retardation due to malnutrition Therefore the group probably represents the lower part of the Gaussian distribution of birthweight but fails to show the signs which have been called the earliest biochemical abnormality of malnutrition (21) The fact that birthweight falls below the 10th percentile for gestational age does not necessarily imply that foetal growth has been impaired by foetal malnutrition or disease involving placental function This seems to be important to keep in mind when investigating population samples of newborn infants defined by anthropometric measurements only

ACKNOWLEDGEMENT

Work supported by the Nestle Foundation Adiopodoumé (Ivory Coast) and Lausanne (Switzerland)

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Submitted April 18, 1979
Accepted Aug 14, 1979

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Acta Pædiatr Scand 69 205–210, 1980

PLASMA SECRETIN IN NEONATES

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ABSTRACT Lucas, A , Adrian, T E , Bloom, S R and Aynsley Green, A (University Department of Paediatrics, John Radcliffe Hospital, Oxford and Hammersmith Hospital, ... neonates Acta Paediatr Scand, 69 205, 1980.—Plasma secre-

... declined towards adult values In contrast, p...

adults (p<0 001), but subsequently declined towards adult values ... who had not been fed for the first 6 days of life, had presistently high basal plasma secretin values In term infants at 6 days of age and in preterm infants up to 13 days, there was no secretin response to a feed However, by 24 days, preterm infants showed a marked postprandial secretin elevation (p<0 02) No correlations were found between plasma secretin concentrations and either blood glucose or plasma insulin concentrations following a feed Significant adjustments in plasma secretin levels occur in the early weeks of life which may be influenced by enteral feeding

KEY WORDS Plasma secretin, neonate, infant feeding, glucose, insulin

Secretin was the first hormone to be discovered Bayliss & Starling described its role in 1902 (2), but further physiological studies in vivo have been impeded by the lack of satisfactory assay techniques However, since the elucidation of the 27 amino acid sequence of secretin (23) and its subsequent chemical synthesis (4), increasingly sensitive radio immunossays have been developed (12, 28, 30, 35) and during the last 3 years it has become possible to measure small, biologically significant changes in plasma concentrations

Secretin is synthesized in specific endocrine cells (S cells) in the duodenum and jejunum (25 28) and its release is stimulated by acid in the small intestine (5, 21, 24, 31, 34) Of the many diverse actions ascribed to secretin, it is likely that the only one of physiological significance in adults is the stimulation of alkaline pancreatic and biliary juice (13)

Pancreatic exocrine function has been studied in early life (14, 32), because of is relevance to the understanding of cystic fibrosis and there have been several reports of gastric acid production in the immediate postnatal period (6, 8, 15, 18, 22) However, there is little information on the control of secretin release in neonates As part of a larger study on metabolic and hormonal adaptations to extra uterine nutrition, we have measured plasma secretin concentrations in relation to feeding, postnatal age, plasma insulin and blood glucose in over 250 term and preterm neonates

PATIENTS AND METHODS

Ninety six normal term infants (birthweight 3292±53 g mean ± S E M and gestation 39 5±0 1 weeks) and 158 healthy preterm infants (birthweight 1968±32 g gestation 33 5±1 weeks) were studied with the approval of the Oxford Area Ethics Committee Each infant in the study contributed only one venous blood sample, either at birth (venous cord blood at vaginal delivery) or postnatally Postnatal samples were obtained from the back of the hand using an open needle technique and were taken only at a time when blood was alos required for clinical mon...

... group and a formula fed group (Cow & Gate premium), were studied on the sixth day of life, when a blood sample was required for the routine Guthrie test Both breast fed

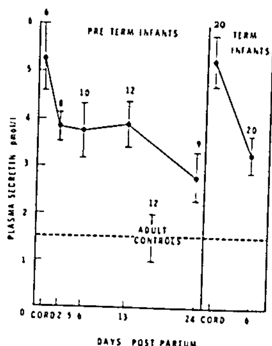


Fig. 1. Postnatal changes in basal plasma secretin concentrations (pmol/l ± S.E.M.) in healthy term and preterm infants, compared with fasting adult controls.

and formula-fed infants received 4-hourly feeds which were completed within 25–35 min. The mean feed volume (20 ml/kg) was similar in the two groups: the breast-fed infants' intake was estimated by test weighings on a sample of 30 feeds. Blood samples were taken either before or at 55, 90 or 150 min after the start of the feed. 8–12 samples were taken for each time period.

Preterm infants were studied in four postnatal groups at mean ages of 2½ (range 1–4), 6 (5–7), 13 (9–17) and 24 (18–42) days. These infants were fed human milk by nasogastric tube over five min as described previously (1). Infants 2½ days old were fed 2-hourly, the other groups 3-hourly. Mean feed volumes were 8 ml/kg, 21.5 ml/kg, 22.5 ml/kg, and 22.5 ml/kg respectively. Blood samples were taken either before or at 30, 60 or

Table 1. *Effect of feeding on plasma secretin in breast- and formula-fed (Cow & Gate premium) term infants, pmol/l±S.E.M.*

	Time after start of feed, minutes			
	0	55	90	150
Formula-fed	3.3 ±0.6 n=10	3.3 ±0.5 n=8	2.8 ±0.5 n=8	2.8 ±0.5 n=8
Breast-fed	3.3 ±0.5 n=10	3.5 ±0.4 n=12	3.4 ±0.6 n=12	2.2 ±0.4 n=8

Table 2. *Effect of feeding on plasma secr[etin] in preterm infants, pmol/l±S.E.M.*

2½-day-old infants fed 2-hourly, 6- and 13-day-old in[fants] fed 3 hourly

Mean post natal age, days	Time after start of feed, min[utes]			
	0	30	60	120
2½	3.8 ±0.3 n=8	3.5 ±0.7 n=8	4.7 ±0.5 n=9	-
6	3.7 ±0.6 n=10	4.8 ±0.5 n=10	4.2 ±0.4 n=12	3.6 ±0.4 n=[illegible]
13	3.9 ±0.5 n=12	4.5 ±0.5 n=8	3.9 ±0.5 n=13	3.5 ±0.7 n=[illegible]

120 minutes after the beginning of the feed. 8–13 sam[ples] were taken for each time period. All preterm infants w[ere] nursed in incubators at environmental temperatures [ap]propriate to their weight and gestation.

infants required ventilatory assistance and enriched [in]spired oxygen concentrations, but none was hypo[xic] (P_{aO_2} maintained between 60–90 torr) or acidotic ([pH] above 7.25) at the time of the study.

Umbilical cord venous blood was taken from 20 te[rm] and 6 preterm neonates delivered vaginally: none h[ad] evidence of foetal distress or birth asphyxia.

Venous blood samples (2 ml) were collected in

porcine secretin and which detected differences be[tween] individual samples of 1 pmol/l with 95% co[n]fidence (11). To avoid interassay variation, all sampl[es] were analysed in the same assay, which also contain[ed] control' blood from 12 healthy adults who had fasted f[or] 4 hours.

Statistical analyses were performed using the Mann Whitney rank sum test (using a two-tailed test of sig[ni]ficance), unless otherwise stated.

RESULTS

Term ($n=20$) and preterm ($n=6$) cord plasma secretin levels were similar; 5.3±0.6 pmol/l (mean ± S.E.M.) and 5.3±0.7 respectively. In contrast, the concentration in 12 healthy fasting adults was 1.5±0.5 pmol/l, signifi-

IMPORTANT

Change of Reference Style

From January 1981, Acta Paediatrica Scandinavica will change its style for citing references and adopt the "Vancouver style" In all manuscripts submitted after June 1, 1980 references should be numbered consecutively in the order in which they are first mentioned in the text Identify references in text, tables and legends by arabic numerals (in parenthesis) Correct forms of references

Standard Journal Article

(list all authors when six or less, when seven or more, list only first three and add et al)

1 Smith GP, Peterson AC, Brown ML Coarctation of aorta Acta Paediatr Scand 1979, 68 315–21

Chapter in Book

2 Smith LW, Carlsson C Neonatal hyperbilirubinaemia In Brown DF, Ericsen O, eds The physiology of the newborn infant 5th ed Stockholm Almqvist & Wiksell, 1974 193–201

Book

3 Jones G Textbook of paediatrics Uppsala. Almqvist & Wiksell, 1978

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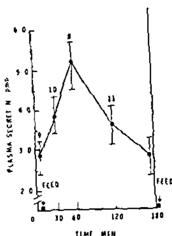


Fig 2 Plasma secretin response to a milk feed in preterm infants at 24 days of age (mean ± S E M) The data bar at 0 has been inserted again at 180 min just prior to the next feed so that the changes during the complete feed cycle are demonstrated

cantly lower than either cord value ($p<0\ 001$, $p<0\ 001$) Fig 1

In term infants on the sixth day, the mean basal plasma secretin concentration was 3 3± 0 6 pmol/l in breast fed and 3 3±0 6 in formula fed infants, overall mean for both groups, 3 3±0 4 pmol/l a significant fall from cord levels ($p<0\ 02$ Fig 1) No change in plasma secretin occurred in either group after feeding (Table 1)

In preterm infants the mean basal secretin level had fallen by 24 days to 2 8±0 5 pmol/l, a value significantly lower than the cord level ($p<0\ 02$ Fig 1) At mean ages of 2½, 6 and 13 days there was a tendency to postprandial elevation but these increments were not significant (Table 2) by 24 days, however, there was a marked postprandial rise from 2 8±0 5 pmol/l ($n=9$) to 5 1±0 6 pmol/l ($n=8$) at 60 min ($p<0\ 02$ Fig 2)

Sixth day preterm infants who had never been fed enterally had a mean plasma secretin level of 5 2±0 6 pmol/l, which was significantly higher than the sixth day basal level of 3 7±0 6 pmol/l in fed preterm infants ($p<0\ 05$), and similar to the cord level

There was no correlation between plasma secretin concentrations and either blood glucose or plasma insulin concentrations at the time of the peak postprandial insulin response in either term infants (at 55 min) or preterm infants (at 30 min) (Table 3)

Table 3 *Relationship of postprandial plasma secretin concentrations to blood glucose and to plasma insulin concentrations in term infants (55 min after start of feed) and preterm infants (30 min)*

Glucose	Insulin
Term	
$r=-0\ 22$	$r-0\ 05$
$n-14$	$n=19$
p=N S	p=N S
Preterm	
$r=0\ 13$	$r-0\ 17$
$n=40$	$n=48$
p=N S	p-N S

DISCUSSION

At birth, plasma secretin concentrations in both term and preterm healthy infants are over three times higher than those found in adults, these levels subsequently decline during the neonatal period High secretin concentrations in cord blood have been described once before (29), though in this report a postnatal rise was observed However, the concentrations noted in that study were several times higher than those in our study, in which the adult concentrations were similar to those reported by others (12, 30) It seems unlikely that the high level of plasma secretin at birth could be explained by acid in the duodenum since there is evidence that the pH of the stomach contents at delivery is around neutral (6, 8, 22), reflecting the pH of swallowed amniotic fluid (15) Another possible explanation is that raised plasma secretin relates to the lack of enteral feeding in newborn infants at birth state, since starvation has been proposed as a stimulus to

secretin release in adults (16), though others have disputed this (10, 13) Reduced secretin clearance in the neonate must also be considered Secretin is normally rapidly removed from plasma by liver and kidneys in adult dogs (7, 20), and has a half-life of around 3 min in adult man (12), though little data is available on the human fetus at birth A further possibility is that the raised plasma secretin levels could be accounted for by a higher secretin cell mass at birth, but this has not been established in man

We have considered that in addition to its role in stimulating the exocrine pancreas, the high levels of secretin play a unique role in metabolic and physiological adaptations in the perinatal period In pharmacological doses in adults, secretin exerts diverse effects including stimulation of cardiac output and splanchnic blood flow, lipolysis, glycolysis and cyclic AMP production (13) It is conceivable that the threshold for some of these effects is lower in neonates, but clearly further work is needed to evaluate this proposition

It was of interest to find that the decline in secretin which occurred during the first 6 days of life in term and preterm infants did not occur in sixth day preterm infants who had not been fed on account of hyaline membrane disease The failure of plasma secretin to fall in these unfed infants could have been due either to their underlying respiratory disease, or to the lack of enteral feeding If the latter was the causative factor, then it is possible that early enteral feeding is important in regulating basal secretin concentrations The role of gastric acid in the control of basal secretin levels in the neonatal period remains to be established Intragastric pH has been shown to decline rapidly in the first 6–8 hours after birth, then rise more slowly and then decline again in the second week of life (8, 15, 18) Thus, the relationship between gastric acid production and secretin release is likely to be complex

Our data demonstrate that a plasma secretin response to feeding is not present in term infants by the sixth day and does not occur in preterm infants until after 3 weeks of age when there is a nearly 100% elevation one hour after the feed. The secretin response to feeding in adults has been much disputed (13) and some authors have failed to find a consistent elevation after a meal (9, 19, 27), perhaps because of the variability and transience of response in individual subjects (31) However, with improved sensitivity of the radioimmunoassay definite though small elevations in plasma secretin may be found reliably after certain types of meal (13) The plasma secretin response in adults is much less after food than with acid stimulation because of the high pH of most foods, the relatively slow release of food from the stomach into the duodenum and the fact that the threshold stimulus for secretin release is a duodenal pH <4 5 (11) As might be expected, acidic drinks cause a substantial plasma secretin elevation in adults (13) and children (3) Since the pH of milk consumed by the infants in our study was around 7 0 (6 9 for Cow & Gate premium, >7 1 for banked human milk), this could have been a significant factor in the absence of secretin response in the first 3 weeks of life However the substantial response in the 24-day-old preterm group required explanation It is possible that this was due to the increasing efficiency of gastric emptying which occurs during the early weeks of life Alternatively the duodenal and jejunal S cells, though capable of a high basal output of secretin, may not become responsive to stimuli until some maturation process has occurred It is nevertheless paradoxical that in the first week of life feeding appeared to depress basal secretin levels, whereas later, beyond 3 weeks of age feeding causes an increase in plasma secretin It is possible that this difference could be related to progressive changes in the release of other gastrointestinal hormones which we have found in the neonatal period (unpublished)

Finally, we have examined the relationship of plasma secretin to postprandial insulin and

…e levels, since there have been several …s suggesting that secretin may be an …or in the enteroinsular axis in adults (33), …h others believe this to be a phar…logical effect (13, 17). Buchanan et al. …ted an inverse relationship between …se and secretin in neonates which they …uted to insulin release (29). We were not …to find any relationship between plasma …tin and blood glucose of plasma insulin …wing a feed in either term or preterm …ts (Table 3).

CONCLUSION

…e significant findings emerge from these …es: firstly, that secretin levels are high in …mmediate neonatal period, compared with …lts. Secondly, that the postnatal fall in …etin seen in 6-day old healthy preterm …nts is not seen in infants who have been …rived of enteral feeding, thus suggesting … feeding may play a part in regulating …tnatal changes in the release of this hor…ne. Thirdly, that elevations in plasma se…tin after feeding do not emerge until after …eeks of age in preterm infants. These data …vide further insight into the physiological …aptations to feeding which occur in infants …er birth.

ACKNOWLEDGEMENTS

… are grateful to the nursing staff of the Special Care …by Unit, John Radcliffe Hospital, Oxford and to Pro…ssor J. P. M. Tizard for his advice and encouragement. … L. is in receipt of a Wellcome Research Fellowship.

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Submitted March 27, 1979
Accepted Aug 6 1979

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Acta Pædiatr Scand 69 211–214, 1980

PLASMA PANCREATIC POLYPEPTIDE IN THE HUMAN NEONATE

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ABSTRACT Lucas, A , Adrian, T E , Bloom, S. R and Aynsley Green, A. (University Department of Paediatrics, John Radcliffe Hospital, Oxford and Hammersmith Hospital, London) Plasma pancreatic polypeptide in the human neonate. Acta Paediatr Scand, 69 211, 1980.—Plasma pancreatic polypeptide (PP) concentrations have been studied in 197 healthy term and preterm infants At birth plasma concentrations were lower than those found in the young fasting adult ($p<0\ 01$), but by the sixth postnatal day in both term and preterm infants basal concentrations had risen to adult levels In preterm infants, who were studied at two further postnatal ages, 13 and 24 days, basal plasma PP concentrations peaked at 13 days with levels that were over twice those seen in fasting adults ($p<0\ 005$) However, the marked PP elevations following feeding that have been reported in adults, were not seen in any of the groups of neonates studied PP physiology thus appears to differ in neonates and adults These findings may be relevant to adaptation to postnatal life

KEY WORDS Plasma pancreatic polypeptide (PP), neonate, infant feeding

Human pancreatic polypeptide (PP) was discovered incidentally during the investigation of impurities in insulin preparations (9) In primates it appears to be released exclusively from specific endocrine cells in the pancreas (1 8) In adults PP is secreted in large amounts in response to food (2, 11, 15) and an entero-PP axis has been postulated to account to its release (2) Although the physiological importance of PP has not yet been defined, it is thought that it may play a role in inhibiting pancreatic enzyme secretion and gall bladder contraction (7) However, not until now has PP been studied in the human neonate In order to shed more light on the development of pancreatic endocrine function, we have measured plasma levels of this peptide in 197 term and preterm infants

PATIENTS AND METHODS

Forty three normal term infants (mean birthweight 3290±50 (S E M) g and gestation 39 5±0 1) and 128 healthy preterm infants (1970±30 g and 33.5±0 1 weeks) were studied with the approval of the Oxford Area Ethics Committee Each infant contributed only one venous blood sample taken at the same time as blood was required for routine clinical purposes

The term infants were studied at 6 days of age and were breast fed 4-hourly over 25–35 min with a mean feed volume of 20 ml/kg Blood samples were taken either before a feed or at 55, 90 or 150 min after the beginning of the feed

Preterm infants were either 6 (range 5–7), 13 (11–17) or 24 (18–42) days old and were fed 3 hourly using pooled human milk given by nasogastric tube over 5 min (10) (mean feed volume of 22 ml/kg)

Blood was taken either before a feed or at 30, 60 or 120 min after the feed These infants were nursed in incubators at environmental temperatures appropriate to their weight and gestation None had any clinical problems other than prematurity

A further group of 10 preterm infants were studied (mean age 6±0 5 days, birthweight 1875±243 g, gestation 33±1 weeks) who had received only intravenous dextrose since birth on account of inability to tolerate enteral feeds because of hyaline membrane disease These unfed infants required ventilatory assistance and enriched inspired oxygen concentrations, but none was hypoxic (P_{aO_2} maintained between 90–60 torr) or acidotic (pH above 7 25) at the time of the study

In addition, umbilical venous blood was taken at vaginal delivery from 20 term and 6 preterm healthy infants, and control blood taken from 12 healthy fasting adults (age range 22–34 years)

Venous plasmas were assayed for PP using a sensitive and specific radioimmunoassay with an antibody (615–R110–146–6) raised to human PP and human PP standards and label Pooled cord plasma was tested in the

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Submitted March 27, 1979
Accepted Aug 6, 1979

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Table 1 *Plasma pancreatic polypeptide (pmol/l±S E M) response to feed on sixth natal day*

infants				
e minutes	0	55	90	150
	28 ±6 *n* 10	30 ±5 *n*–12	21 ±3 *n*=12	32 ±6 *n*=9
term infants				
ie minutes	0	30	60	120
	43 ±9 *n* 10	30 ±9 *n*=11	43 ±14 *n*=12	30 ±5 *n*=13

Greenberg et al have shown a stimulatory fect of pharmacological doses of PP on pan eatic DNA synthesis, and it is therefore ssible that the postnatal elevation of basal P may be important in the adaptation to xtra uterine life, by stimulating pancreatic rowth (6)

In adults the PP response to feeding is npressive Fat and protein exert the greatest timulatory effects (4) and these potentiate ach other Following a mixed meal, given to asting healthy adults, there is a prompt ten- old elevation in plasma PP, which is main- ained for over 6 hours It is thought that this elevation is brought about by both hormonal and vagal influences (2 11) In our studies we were unable to demonstrate a rise in PP after a milk feed in breast fed term infants on the sixth day or in preterm infants up to 24 days of age In fact on the thirteenth day, when basal levels were highest, preterm in fants showed an actual fall in PP 30 min after the feed, suggesting that PP output may be suppressed by feeding at this age At no point in the neonatal period were we able to demon strate levels of PP after feeding which reached even half the adult postprandial PP levels re ported by this laboratory It is possible, how- ,er, that there had been a transient PP eleva- before our first postprandial sampling at 30 min

A possible explanation for the absence of postprandial plasma PP elevation is that the short feed to feed interval in neonates (3–4 hours) prevents a true basal level of PP being achieved In adults the postprandial plasma PP concentrations remain elevated above basal, though declining for more than 6 hours We have not, however, observed lower PP levels in a group of term infants who were fed 6-hourly than those who were fed 4-hourly (unpublished) Alternatively, the lack of PP elevation following a feed in neonates compared with adults, could be due to relative insensitivity of PP release mechanisms or be secondary to absence of appropriate stimuli Cholecystokinin and gastric inhibitory polypeptide (GIP) cause PP release, whereas somatostatin is inhibitory (3, 4) We have not studied cholecystokinin or somatostatin in neonates, but we have found in preterm infants that, while basal GIP levels increase during the early weeks of life, the phasic GIP response to feeding is not seen until the fourth week (unpublished), and this could contribute to the absence of a rise in postprandial PP output Since PP is an inhibitor of exocrine pancreatic and biliary secretion (7) it is possible that the lack of PP response to feeding is 'adaptive', allowing maximum stimulation of exocrine secretions in the neonatal period Thus it is probable that the entero-PP-axis does not operate fully in the early weeks of life

Further studies are needed to define the role of PP in the neonatal period—in particular to establish whether this peptide exerts a significant trophic influence on pancreatic growth and whether the absent responses to feeding in early life have important physiological consequences

ACKNOWLEDGEMENTS

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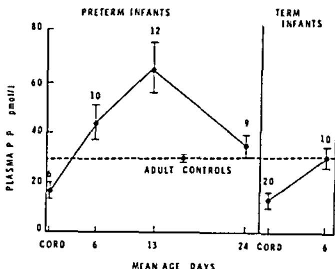


Fig 1 Basal plasma pancreatic polypeptide (PP) (pmol/l±S E M) in preterm (*left*) and term (*right*) infants in venous cord blood and postnatally , represents mean plasma PP concentration in healthy young fasting adults

standard curve where it behaved in a manner identical with pooled normal adult plasma This assay showed no cross reaction with other gut hormones and was capable of detecting changes of 4 pmol/l with 95% confidence (1)

Statistical analyses were performed using the non parametric Mann Whitney rank sum test but means and standard errors are shown for comparison

RESULTS

Mean umbilical venous plasma levels were similar in term and preterm infants at 13±3 and 17±3 pmol/l (mean±S E M) These levels were significantly lower than the young healthy fasting adults values of 28±1 ($p<0.01$)

In term infants the mean basal level on the sixth day was 28±6, significantly higher than the cord value ($p<0.05$), and similar to the adult value (Fig 1) There was no change in PP level following a feed (see Table)

In preterm infants, the basal PP concentration rose from the cord level to 43±9 on the 6th day ($p<0.02$) and to 65±11 on the 13th day ($p<0.005$), which was significantly higher than the adult level ($p<0.005$), but subsequently it fell to 34±5 at 24 days ($p<0.05$) (Fig 1) There were no significant postprandial changes in PP at 6 days, but at 13 days there was a significant fall at 30 min ($p<0.02$) (Fig 2*a*) and at 24 days there was a small and non significant elevation (Fig 2*b*)

The group of preterm infants with hyaline membrane disease who had not been fed enterally from birth had a mean plasma PP concentration of 41±8 pmol/l

DISCUSSION

At birth, plasma concentrations of plasma pancreatic polypeptide were found to be low compared with the fasting adult level This observation is consistent with the demonstration by Floyd et al of increasing plasma PP levels in adults with advancing age (5) However, after birth in both term and preterm infants there was a sharp rise in basal plasma PP and in 13 day old preterm infants, there was a peak basal concentration which significantly exceeded the level in young adults We have observed a postnatal surge in basal levels of three other gut hormones—motilin gastrin and enteroglucagon—and have suggested that these hormone changes may be induced by enteral feeding (submitted for publication) The results of the present study show that in contrast, the increase in basal PP levels by the sixth day of life in both preterm and term infants, was also seen in preterm infants who had not received enteral feeds It seems therfore that this elevation of PP may not be due to enteral feeding itself

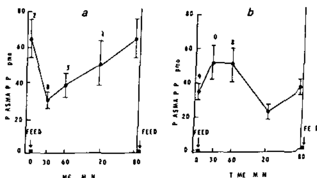


Fig 2 Change in plasma pancreatic polypeptide (pmol/l± S E M) after a nasogastric feed of human milk in preterm infants at (*a*) 13 days and (*b*) 24 days after birth *n* values are shown above the mean and standard error range bars The zero observations are shown also at 180 min prior to the next feed

Table 1 *Plasma pancreatic polypeptide (pmol/l±S E M) response to feed on sixth postnatal day*

Term infants				
Time minutes	0	55	90	150
	28 ±6 n−10	30 ±5 n=12	21 ±3 n=12	32 ±6 n−9
Preterm infants				
Time minutes	0	30	60	120
	43 ±9 n=10	30 ±9 n=11	43 ±14 n=12	30 ±5 n 13

Greenberg et al have shown a stimulatory effect of pharmacological doses of PP on pancreatic DNA synthesis and it is therefore possible that the postnatal elevation of basal PP may be important in the adaptation to extra uterine life, by stimulating pancreatic growth (6)

In adults the PP response to feeding is impressive Fat and protein exert the greatest stimulatory effects (4) and these potentiate each other Following a mixed meal, given to fasting healthy adults, there is a prompt ten fold elevation in plasma PP, which is maintained for over 6 hours It is thought that this elevation is brought about by both hormonal and vagal influences (2, 11) In our studies we were unable to demonstrate a rise in PP after a milk feed in breast fed term infants on the sixth day or in preterm infants up to 24 days of age In fact on the thirteenth day, when basal levels were highest, preterm infants showed an actual fall in PP 30 min after the feed, suggesting that PP output may be suppressed by feeding at this age At no point in the neonatal period were we able to demonstrate levels of PP after feeding which reached even half the adult postprandial PP levels reported by this laboratory It is possible, however, that there had been a transient PP elevation before our first postprandial sampling time at 30 min

A possible explanation for the absence of postprandial plasma PP elevation is that the short feed to-feed interval in neonates (3–4 hours) prevents a true basal level of PP being achieved In adults the postprandial plasma PP concentrations remain elevated above basal, though declining for more than 6 hours We have not, however, observed lower PP levels in a group of term infants who were fed 6-hourly than those who were fed 4-hourly (unpublished) Alternatively, the lack of PP elevation following a feed in neonates compared with adults, could be due to relative insensitivity of PP release mechanisms or be secondary to absence of appropriate stimuli Cholecystokinin and gastric inhibitory polypeptide (GIP) cause PP release, whereas somatostatin is inhibitory (3, 4) We have not studied cholecystokinin or somatostatin in neonates, but we have found in preterm infants that, while basal GIP levels increase during the early weeks of life, the phasic GIP response to feeding is not seen until the fourth week (unpublished), and this could contribute to the absence of a rise in postprandial PP output Since PP is an inhibitor of exocrine pancreatic and biliary secretion (7) it is possible that the lack of PP response to feeding is 'adaptive', allowing maximum stimulation of exocrine secretions in the neonatal period Thus it is probable that the entero-PP-axis does not operate fully in the early weeks of life

Further studies are needed to define the role of PP in the neonatal period—in particular to establish whether this peptide exerts a significant trophic influence on pancreatic growth and whether the absent responses to feeding in early life have important physiological consequences

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Submitted May 31, 1979
Accepted Aug 6 1979

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Acta Pædiatr Scand 69 215–218, 1980

SERUM GROUP I PEPSINOGENS IN CHILDREN

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ABSTRACT Waldum, H L , Straume, B K , Burhol, P G and Bredrup Dahl, L (Laboratory of Gastroenterology, Department of Medicine and Department of Paediatrics, University Hospital of Tromsø, and Institute of Community Medicine, Tromsø, Norway) Serum group I pepsinogens in children Acta Paediatr Scand, 69 215, 1980 —Serum group I pepsinogens (PG I) were determined by a radioim n noassay method in blood drawn from premature and newborn infants, children of variou

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tures and newborns, and there was

and their newborn full term infants

but remained significantly reduced up to the age of 10 years These findings are in agreement with those reports showing an increasing pepsin secretion during childhood, and thus indirectly lend support to the use of serum PG I as an estimate of gastric pepsin secretion

KEY WORDS Children, newborns, prematures, serum group I pepsinogens

The incidence of peptic ulcer disease in children increases with increasing age up to puberty (3, 18) This agrees well with previous reports claiming reduced gastric acid and pepsin outputs per kg body weight up to the age of 11 years, and particularly during the first 2 years of life (1, 10) By contrast, serum pepsin activity which is an indirect estimate of gastric acid and pepsin secretion (7, 9), has been reported to be increased during the first week of life (6) Furthermore, a correlation between serum pepsin activity in the mother and that in her newborn child has been found (22), and the elevated serum pepsin activity in the first week of life as compared with older children (6), could possibly be explained by transfer of pepsinogens across the placenta

The seven pepsinogens found in human gastric mucosa (12) may be divided immunologically into two groups (PG I and PG II) (13) Since PG I is produced only in the oxyntic area of the stomach (14), the determination of serum PG I by a radioimmunoassay method (15) may be assumed to give a reliable estimate of gastric secretory capacity, which, actually has been shown both for gastric acid (16, 19) and gastric pepsin (19) secretion This study was therefore undertaken to assess serum PG I in children

MATERIALS AND METHODS

Blood was drawn from 18 women during delivery and from the umbilical cord of their babies as well as from 72 non fasting children including 5 prematures admitted to the hospital Children with gastrointestinal complaints reduced kidney function or severe illnesses were excluded A control group of 22 healthy non fasting students was also included

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method with a detection limit of 2 6 *ng/ml* an intra assay variation of 6% and an inter assay variation of 17% (20) To avoid a higher inter assay *variation* vis à vis the intra assay variation all samples were analysed simultaneously

The samples were grouped according to age (Table 1) *Group I* prematures [blood drawn *from* prematures 1–4 weeks after birth with a mean biological age (time since conception) of 36 weeks (range 33–39 weeks) and mean weight of 1885 g at *blood sampling*] *Group II* newborns

Table 1 *Serum group I pepsinogens (PG I) in children, healthy students and women at delivery*

Group no	Group	*n*	Mean age ± S E M (years)	Mean serum PG I ± S E M (ng/ml)
I	Premature infants	5	36/52±1/52 (biological age)	22± 2
II	Newborn infants (blood from umbilical cord)	18	40/52±0/52 (gestational age)	26± 2
III	Infants less than 1 year old	6	0 5±0 1	77± 5
IV	Infants 1–2 years	26	1 6±0 1	98± 8
V	Children 3–6 years	14	4 9±0 4	92±12
VI	Children 7–10 years	13	9 4±0 2	95± 8
VII	Children 11–14 years	8	12 6±0 3	107±11
VIII	Students 19–38 years	22	22 3±1 3	133± 9
IX	Women at delivery	18	27 5±1 3	127±11

[blood drawn from the umbilical cord of full term children with a mean gestational age of 40 weeks (range 38 to 42 weeks)], *Group III* infants less than 1 year old [prematures and newborns excluded], *Group IV* children 1 to 2 years old, *Group V* children 3–6 years old, *Group VI* children 7–10 years old, *Group VII* children 11–14 years old, *Group VIII* healthy students 19–38 years old, and *Group IX* women at delivery (mothers of group II) The distribution of the serum PG I values in the different groups was tested by Shapiro Wilks normality test (17), and the group differences in mean serum PG I were evaluated by a one way analysis of variance The correlation between serum PG I in women at delivery and their newborn infants was evaluated by the correlation coefficient *r*

RESULTS

The mean age and mean serum PG I concentration in the different groups are given in Table 1 Normal distribution of serum PG I was achieved in all groups by transformation to the natural logarithm This transformation also equalized the variance in the different groups with a coefficient of variation of about 10% The mean serum PG I value in prematures and full-term newborns were significantly lower than that found in the other children (groups III, IV, V, VI and VII) and the control group of healthy adults (group VIII) On the other hand, there was no significant difference between mean serum PG I in prematures (group I) and full-term newborns (group II) However, a highly significant difference was found between mean serum PG I in newborns (group II) and their mothers (group IX), and there was no correlation between serum PG I in these groups ($r=-0\ 15$)

The difference in mean serum PG I in children (prematures and newborns excluded) and young adults (group VIII) was highly significant ($p=0\ 0002$) Significant differences between mean serum PG I in the separate groups III, IV, V and VI on the one hand and the control group VIII on the other were also found On the other hand, there was no significant difference between mean serum PG I in the group of older children (group VII) and young adults (group VIII) ($p=0\ 18$) Neither was there any significant difference between mean serum PG I in the various groups of children (groups III, IV, V, VI and VII) (prematures and newborns excluded)

DISCUSSION

Our findings concerning serum PG I tally with earlier reports on gastric H^+ and pepsin secretion in children (4) Thus, Werner (21) found only a few pepsinogen granules in the gastric mucosa of human fetuses less than 2 300 g, and more recently Ahmed et al (2) reported peptic activity in the gastric juice first 34 weeks after conception In agreement with these observations, we also found very low serum PG I values in the 5 prematures examined The biological ages (time since conception) of these infants at blood sampling ranged between 32

and 39 weeks, and there was no apparent correlation between age and serum PGI value in these 5 prematures On the contrary, as also can be seen from the low standard error of the mean, serum PGI was very similar in all the prematures and the full term newborns Unfortunately, since our group of prematures is too small and with a mean age too close to full term newborns, we cannot assess the interesting possibility of using serum PGI in the evaluation of fetal maturity However, in a recent report Liebman & Samloff (8) reported significantly reduced serum PGI in prematures 31–36 weeks of gestational age as compared with full term infants

As already mentioned, we found little (but not a complete lack of) PGI in serum of full term newborns This tallies closely with the report of Agunond et al (1) who studied gastric H^+ and pepsin outputs after betazole stimulation in small children As in our findings on serum PGI, they found low outputs of gastric H^+ and pepsin on the first day of life On the other hand, our findings apparently contrast with those of Grayzel et al (6) who found higher peptic activity in the serum of newborns than in older children The apparent discrepancy is hard to explain, but can most likely be ascribed to methodological differences

The lack of any correlation between serum PGI in women at delivery and serum PGI in their newborns, seems to preclude that PGI is transported from the mother to the fetus across the placenta Thus the serum PGI found in fetuses would seem to be produced in their own stomachs, and so may be assumed to reflect the function of their gastric mucosa This seems to contrast with a prior report (22), showing a correlation between serum peptic activity in mother and child

Mean serum PGI increased significantly between birth and an average age of 6 months (ranging nearly up to 1 year) This corresonds to the increase in gastric acid and pepsin secretion observed during the first months of life (1) However, in spite of this increase, lower gastric acid and pepsin outputs per kg body weight were observed in 3-month old infants than in healthy adults (1) We also found significantly lower mean serum PGI values in the group of infants less than 1 year old than in the control group Similarly, Rødbro et al (10) reported lower gastric acid secretion per kg body weight in a group of infants with a mean age of 17 months than in adults, which corresponds to our finding of a significantly lower serum PGI value in the infants 1–2 years old than in the controls Furthermore, Rødbro et al (11) in another communication reported a significant correlation between age and basal gastric pepsin secretion in small children, but not between age and histamine stimulated pepsin secretion We also found significantly reduced serum PGI in all groups of children—apart from group VII, which consisted of older children with a mean age of 12 6 years The mean serum PGI in this group fell between the values obtained in the other groups of children and the value of the adult control group Since a marked rise in serum PGI apparently occurred at puberty, it seems reasonable to assume that hormonal changes cause this increase

In conclusion, our study on serum PGI in children corresponds with prior reports on gastric acid and pepsin secretion Furthermore, the observed rise in serum PGI with age in children corresponds to an increasing frequency of peptic ulcer disease up to (3) and particularly after puberty

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Submitted March 30 1979
Accepted Aug 9, 1979

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Acta Pædiatr Scand 69 219–224, 1980

ENTEROTOXIGENIC AND INVASIVE ESCHERICHIA COLI AS CAUSES OF CHILDHOOD DIARRHOEA IN FINLAND

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ABSTRACT Maki, M , Vesikari, T and Gronroos, P (Departments of Paediatrics and Microbiology, Tampere Central Hospital, Tampere, Finland) Enterotoxigenic and invasive *Escherichia coli* as causes of childhood diarrhoea in Finland Acta Paediatr Scand, 69 219, 1980.—*E coli* was considered as the possible aetiologic agent in 16 cases (5 7%) of 283 hospital admissions for diarrhoea One invasive strain was isolated from a case with exudative diarrhoea Four heat labile (LT) enterotoxin producing strains were found in relatively mild cases of diarrhoea Eleven strains belonged to "classic" pathogenic serotypes (EPEC), 9 of these were endemic cases and 2 associated with travel Of the latter, 1 strain (078) was also found to produce heat stable (ST) enterotoxin detectable by infant mouse assay Although EPEC are now found much less frequently than 20 years ago, *E coli* as a whole may still be the most common bacterial aetiology of childhood diarrhoea in Finland

KEY WORDS *E coli*, gastroenteritis, diarrhoea, enterotoxigenic

In the 1950 s enteropathogenic serotypes of *Escherichia coli* (EPEC) were frequently found in endemic diarrhoea of infants in Finland (10 25) and in Sweden (16) There is no recent information regarding the occurrence of the classic EPEC in Finland, in Sweden they have been found only sporadically (1) This is in contrast with the situation in many other countries (5, 12, 15)

The actual pathogenesis of EPEC diarrhoea still remains obscure *E coli* is now known to cause diarrhoea by at least two major mechanisms 1) production of enterotoxins and 2) invasion of the epithelial cells of the gut (6) Two different toxins have been identified The first, which resembles the enterotoxin of *Vibrio cholerae* is a heat labile (LT) protein identifiable in vitro by its action on mouse adrenal cells in culture (4, 26) The second toxin is heat stable (ST), non immunogenic, has a molecular weight of less than 10000, and can only be detected by in vivo tests, usually an assay in infant mice (14, 28) Both enteroinvasive (EIEC) and enterotoxigenic strains of *E coli* (ETEC) belong to certain 0-antigen serogroups, which are different from classic EPEC in most cases, however (21)

There is wide variation in the occurrence of toxigenic *E coli* in childhod diarrhoea series in different studies, ranging from 0 to 86% of cases (28) The importance of ST in human diarrhoea has been questioned (2), but on the other hand some investigators have found it to be of similar significance to LT (7) EIEC appears to be detected only occasionally in childhood diarrhoea (9)

The role of *E coli* as a causative agent of diarrhoea in children has not been much studied as a whole in Scandinavian countries (2) We have now conducted a prospective study of 283 children with diarrhoea in Tampere, Finland From each patient *E coli* was cultured from stool specimens and studied for toxigenicity (LT) and invasiveness In addi-

tion, classic EPEC serotypes were searched for When found, the latter were also tested for the production of ST

MATERIALS AND METHODS

Patients

The study group consisted of all children up to 15 years of age hospitalized for diarrhoea at the Department of Paediatrics Tampere Central Hospital over a one year period from December 1977 to November 1978 There were altogether 283 such admissions Those who in addition to diarrhoea had other symptoms or diseases as well as those who had recently received antibiotics were included in the study Four patients had acquired diarrhoea while being abroad, in addition there was one case possibly associated with travel The remaining cases were considered endemic Nosocomial diarrhoeas (onset 3 or more days after admission) were not included Eighty eight hospitalized children during the same period with no gastrointestinal symptoms served as controls for the bacteriological studies

Collection of specimens

Fresh stool specimens (taken with rectal tube when necessary) were collected for bacterial cultures three times at the acute stage of the diarrhoea The specimens were bedside inoculated in the VMG IV transport medium (20)

Microbiological studies

Each stool specimen was cultured from the transport medium to eosin methylene blue agar plates from which *E coli* was identified by standard methods Five separate colonies from each specimen were cultured and maintained on blood and nutrient agars till further testing Tests for enterotoxin production (LT) and invasiveness were done once or twice a week during the study period and serotyping and tests for ST were done at the end of the study

Serotyping of E coli Each isolate of *E coli* was tested for EPEC as recommended by Farmer et al (8) Presumptive identification of 0 antigens was based on agglutination of living and heated antigen with both Behring Institute Colisera (polyvalent I and II) and Wellcome *E coli* agglutinating sera (polyvalent 2 and 3) followed by agglutination with monovalent antisera The antisera contained the following *E coli* 0 serogroups 026 055 078 086 0111 0114 0119 0124 0125 0126 0127 0128 The results were confirmed by titration of the monospecific 0 antiserum with the presumptive strain

Tests for detection of enterotoxins E coli cultures from blood agars were grown in trypticase soy broth by shaking at 37°C for 24 hours Cultures were centrifuged and the supernatants filtered through a 0 22 μm Millipore® filter The sterile culture filtrates were prepared in the same manner for LT and ST tests

The presence of LT was tested using Y 1 adrenal cells as described by Sack & Sack (26) The LT positive *E coli* strains were retested twice to confirm the results In each test group a known positive LT *E coli* was included as control (strain B 2 C or B 7 A kindly supplied by Samu[el] Formal Walter Reed Army Institute of Resea[rch] Washington D C USA) The adrenal cells were a[...] from Torkel Wadstrom Swedish University of Agri[cul]tural Sciences College of Veterinary Medicine Upps[ala] Sweden

An infant mouse assay was used to detect ST (3) [...] tenth of a millilitre of the sterile culture filtrate with Ev[ans] blue was percutaneously injected into milk filled stoma[chs] of two or three 1 to 4-day-old infant mice (NMR H[a]nover) Four hours later the mice were euthanatized [...] the ratio of gut weight to remaining body weight cal[cu]lated The intestines of all mice in a given test w[ere] weighed together Only mice with visible Evans blue w[...] in the intestines were included A known heat sta[ble] toxin producing *E coli* strain served as positive con[trol] (strain 334 kindly supplied by Richard L Guerrant U[ni]versity of Virginia Charlottesville USA)

Test for invasiveness The invasiveness test was d[one] as previously described (18) In summary human epith[eli]al cells (HEp-2) were cultured on glass cover sl[i]ps [...] nearly confluent monolayers The bacterial suspens[ion] was incubated on the cultures and seven hours later a[fter] washings fixing and staining the HEp-2 cells were m[icro]scopically examined for the presence of intracellula[rly] located bacteria *E coli* strain M4163 from Samuel Formal served as positive control

RESULTS

Diarrhoeagenic *E coli* was cultured from t[he] stools of 16 children (5 7%) From the contr[ol] children no ETEC, EIEC or EPEC were is[o]lated

Invasive strains

An enteroinvasive strain of *E coli* was de[...]tected from only one patient (Fig 1) Th[e] strain was not toxigenic, nor did it belong t[o] the EPEC 0 serogroups tested by us

Case
fever
tion
tive diarrhoea with fresh blood developed on the same da[y]

came symptomless by the fifth day No other en[...]teropathogens were found Acute and convalescent ser[a] one month later were tested for *E coli* antibodies us[i]ng the autologous *E coli* isolate as antigen The correspond[i]ng agglutinin titres were 1 40 and 1 640

Enterotoxigenic (LT) strains

Four patients out of 283 (1 4%) were found to have heat labile enterotoxin producing *E coli*



Fig 1 Invasive strain of *E coli* isolated from an 8-year old patient with diarrhoea (see text) A large number of intracellularly located bacteria can be seen in the cytoplasm of an individual HEp-2 cell

in their stools These *E coli* did not belong to the EPEC serogroups nor did they produce ST There was no history of travel abroad in any of these patients The clinical findings of the patients are summarized in Table 1 In all cases the diarrhoea was relatively mild Three cases were connected with respiratory infections and a concomitant rotavirus infection was found in two cases

Pathogenic serotypes of E coli

Only 11 patients (3 9%) were found to carry EPEC in the stools (Table 2) No EPEC were found in the control group Only one of the strains (from case no 8) produced ST (see later) and all the others were negative for LT and ST The EPEC isolates were not invasive for HEp-2 cells Cases no 8 and 11 were associated with travel (Tunisia), while the remainder had no recent history of travel abroad The clinical findings and the EPEC isolates are shown in Table 2 The severity of the disease ranged from mild to moderate, and watery diarrhoea was characteristic In 4 cases other enteropathogens were also found *Salmonella newington* in one and rotavirus in three

Heat stable enterotoxin producing E coli

All LT *E coli* and EPEC were tested for ST Only one isolate (case no 8 in Table 2) caused fluid accumulation in the infant mouse intestines This ST only producing *E coli* belonged to the serogroup 078

Case report This 18-month-old previously healthy girl acquired diarrhoea while in Tunisia She was in good general condition but had lost 5–6% of her weight upon admission Routine laboratory tests were unremarkable She was first given glucose salt solution perorally, but because of continuing watery diarrhoea the treatment was changed to intravenous fluids Thereafter the diarrhoea ceased within six days having lasted altogether two weeks

DISCUSSION

We have been able to demonstrate bacterial aetiology in 10% of the cases of infantile diarrhoea (19) Diarrhoeagenic *E coli* although infrequent, were the most common bacterial pathogens associated with childhood diarrhoea in our material The other known bacterial enteropathogens in our community are *Campylobacter*, *Salmonella* and *Yersinia*

Invasive *E coli* are rare in childhood diarrhoea (9, 23), and in many studies no cases have been found (7, 12) Our results are in agreement with these reports, as we found only one case among 283 admissions In this patient, both the clinical picture and the laboratory studies—including the presence of leucocytes in the stools (22)—indicated an invasive intestinal pathogen

Enterotoxigenic *E coli* are the most common cause of travellers' diarrhoea (27) In some parts of the world these *E coli* also appear to be important enteric pathogens in

Table 1 *Clinical findings in four cases of LT-E coli associated diarrhoea in children*

Case	Age (months)	Duration of symptoms (days)	Quality of stools	Vomiting	Dehydration at admission	Fever (°C)	ESR	CRP (mg/l)	WBC
1	5	6	Watery	+	None	37 8	9	<4	15 700
2	7	10	Watery	+	Mild	40 0	66	23	16 700
3	11	6	Watery	+	Mild	40 0	6	26	10 500
4	24	11	Watery	+	Mild	40 3	60	16	8 900

children (7, 11, 29), whereas in recent studies in North America they have been rare (12, 23) In our material sporadic endemic cases of childhood diarrhoea were also seldom associated with LT-*E coli* Even in these cases one can question the pathogenic significance of LT *E coli* since the clinical picture was complicated by respiratory infections, antibiotic therapy and simultaneous rotavirus infection

The role of ST-producing *E coli* in childhood diarrhoea is still unclear In a recent epidemiological study in North-America only a few cases of ST *E coli* associated diarrhoea were found (23) The only case of ST *E coli* found in this study was associated with travel We believe that ST *E coli* may not be an important aetiological factor in childhood diarrhoea in Finland either, but further studies are required to confirm this

Only 11 EPEC were found, and 5 of these were of serogroup 078 We have included this serogroup in the list because some authors consider 078 as an enteropathogenic serotype (24), others, however, do not (21) In any case toxigenic *E coli* are often associated with serogroup 078 (17), as was also our ST producing isolate If the five cases of 078 are excluded, the frequency of EPEC in the present study is 2 1% This is in contrast with earlier studies from Finland in the 1950 s Both Gronroos (10) in Turku and Rantasalo & Hallman (25) in Helsinki found EPEC associated with endemic infantile diarrhoea in over 20% of cases In many countries EPEC are still major causative agents of childhood diarrhoea (5, 12 15) Since all our EPEC isolates came from patients with diarrhoea, and no EPEC were found in the control group we believe that EPEC probably were true causative agents of diarrhoea in the present cases We therefore support the opinion that serotyping of *E coli*

Table 2 *Clinical and laboratory findings in 11 cases of enteropathogenic E coli (EPEC) associated diarrhoea in children*

Case	Age (months)	O serogroup	Duration of symptoms (days)	Quality of stools	Vomiting	Dehydration at admission	Fever (°C)	ESR	CRP (mg/l)	WBC
1	4	78	5	Watery	+	None	37 6	6	<4	1000
2	7	78	19	Mucoid	+	Moderate	38 0	12	<4	7 60
3	8	78	15	Watery	+	Mild	39 6	43	61	12 70
4	14	111	6	Watery	+	Mild	39 3	9	18	6 40
5	14	26	18	Loose	+	Mild	40 0	3	12	13 00
6	15	127	11	Watery	+	Moderate	38 1	10	<4	7 400
7	17	126	9	Mucoid	+	Moderate	39 0	5	<4	3 40
8	18	78	15	Watery	−	Moderate	38 2	5	<4	10 70
9	26	78	5	Watery	+	Mild	37 3	3	4	9 00
10	28	125	13	Watery	+	Oedema	37 9	116	<4	9 30
11	79	111	9	Watery	−	Mild	39 0	11	<4	7 80

* URI=upper respiratory tract infection

Associated diseases	Antibiotic treatment at onset of the diarrhoea	Other enteropathogens
Pyelonephritis	Sulfatrimethoprim	None
Otitis	None	Rotavirus
Otitis	Azidocillin	Rotavirus
Otitis pneumonia	Amoxycillin	None

is still a useful diagnostic tool in childhood diarrhoea (13)

In conclusion, the present investigation suggests that diarrhoeagenic *E coli* are not frequent causes of endemic diarrhoea in children in Finland at this time, but as a whole *E coli* still constitutes a significant group among known enteropathogenic bacteria

ACKNOWLEDGEMENTS

This work has been supported by grants from the Finnish National Council for Medical Research, the Emil Aaltonen Foundation and the Foundation for Paediatric Research The technical assistance of Miss Kerttu Saarinen Mrs Taimi Tammipuu Miss Kerttu Kangas and Miss Sinikka Kivistö is gratefully acknowledged

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Associated diseases at onset	Antibiotic therapy at onset	Other enteropathogens
Otitis	Sulfa	*Salmonella newington*
None	None	None
Otitis	Erythromycin	None
URI*	None	Rotavirus
Otitis	Erythromycin	None
URI*	Erythromycin	Rotavirus
None	None	None
None	None	None
URI*	Erythromycin	Rotavirus
Nephrosis	None	None
None	None	None

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Table 1 *Clinical findings in four cases of LT-E coli associated diarrhoea in children*

Case	Age (months)	Duration of symptoms (days)	Quality of stools	Vomiting	Dehydration at admission	Fever (°C)	ESR	CRP (mg/l)	WBC
1	5	6	Watery	+	None	37 8	9	<4	15 [illegible]00
2	7	10	Watery	+	Mild	40 0	66	23	16 [illegible]00
3	11	6	Watery	+	Mild	40 0	6	26	10 [illegible]00
4	24	11	Watery	+	Mild	40 3	60	16	8 900

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Table 2 *Clinical and laboratory findings in 11 cases of enteropathogenic E coli (EPEC) associ diarrhoea in children*

Case	Age (months)	O sero-group	Duration of symptoms (days)	Quality of stools	Vomiting	Dehydration at admission	Fever (°C)	ESR	CRP (mg/l)	WB
1	4	78	5	Watery	+	None	37 6	6	<4	100
2	7	78	19	Mucoid	+	Moderate	38 0	12	<4	76
3	8	78	15	Watery	+	Mild	39 6	43	61	12 7
4	14	111	6	Watery	+	Mild	39 3	9	18	6 4
5	14	26	18	Loose	+	Mild	40 0	3	12	13 0
6	15	127	11	Watery	+	Moderate	38 1	10	<4	7 40
7	17	126	9	Mucoid	+	Moderate	39 0	5	<4	3 4
8	18	78	15	Watery	−	Moderate	38 2	5	<4	10 7
9	26	78	5	Watery	+	Mild	37 3	3	4	9 0
10	28	125	13	Watery	+	Oedema	37 9	116	<4	9 3
11	79	111	9	Watery	−	Mild	39 0	11	<4	7 8

ᵃ URI=upper respiratory tract infection

Acta Pædiatr Scand 69 225–229, 1980

COMPOSITION OF THE INTESTINAL FLUID AND THE FUNCTIONAL JEJUNOILEAL JUNCTION IN SECRETORY DIARRHEA OF CHOLERA IN CHILDREN

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[illegible] D, Johns Hopkins University International Centre for Medical

The study critically evaluates the changes in the chemical composition [illegible] along the whole length of the small intestine in four children with acute cholera and in one with acute noncholeraic diarrhea In the children with cholera the total CO_2 content rose abruptly from a mean of 7.1 mEq/l to 19 6 mEq/l at about two thirds the tube distance from the ligament of Trietz to the end of the ileum and increased further distally. At around the same point the pH also rose and the chloride fell. It is proposed that this level of the small intestine where a sharp transition in total CO_2 content occurs be regarded as the functional jejunoileal junction. Sodium and potassium levels were similar in the jejunum and the ileum and the measured osmolality could be accounted for by them The child with noncholera diarrhea had a very different small intestinal composition i e. the total CO_2 and pH as well as the sodium level remained low while the measured osmolality was high, indicating a high osmotic gap. The presence of a large amount of organic acid anions of bacterial origin and carbohydrate breakdown products may fully explain the findings in this child. More studies, however, are needed on children with noncholera diarrhea to confirm these findings.

KEY WORDS· Children, cholera, jejunoileal physiology, secretory diarrhea, intestinal fluid composition

The secretory diarrhea of cholera is due to the massive outpouring of small intestinal fluid induced by the enterotoxin of V cholerae (1, 2, 3, 10) The fluid thus produced always reflects the normal ionic composition appropriate to the site of its production (1). Hence this fluid is low in bicarbonate and high in chloride in the jejunum while the reverse is true in the ileum (1, 11, 12) The sodium as well as the potassium content and the osmolality, however, remain similar to that of plasma (1, 11, 12) The ionic composition of the cholera stool is determined by the variable modification of the ileal effluent by the large intestine. There are, however, important differences in the ionic composition of faeces in adults and children with cholera. In adults the average concentration of sodium (140 mEq/l) and total CO_2 are higher than those of children while the potassium concentration is lower (11, 12). In noncholera infantile diarrheas (9, 11) the average sodium concentration (56 mEq/l) is nearly half of that in children with cholera (101 mEq/l) (11, 12).

Inspite of the well known functional differences between the jejunum and ileum (6, 7, 13, 15, 17, 18) a satisfactory method to locate the border between these functional zones in vivo is lacking The present study describes the chemical composition of the luminal fluid along the whole length of the small intestine in 4 children with acute cholera and in one

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Submitted Aug 28, 1979
Accepted Oct 8, 1979

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ool composition

Na⁺ (mEq/l)	K⁺ (mEq/l)	Total CO_2 (mEq/l)	Osm (mOsm/kg)	pH
91 2	29 6	37 8	–	7 95
39 0	99 9	29 4	290	7 44
05 5	37 7	34 7	275	7 41
01 5	49 7	49 2	303	7 45
65 3	31 4	15 0	–	–

ured osmolality (measured in three of the four children with cholera) and the derived one i e twice the sum of the sodium and potassium values However, the stool sodium as well as the sum of the sodium plus potassium values were much lower in the only child with non cholera diarrhea (H65) which is in keeping with previous studies on infantile diarrhea (9, 11, 12)

Chemical composition of small intestinal fluid Total CO_2 content rose abruptly from an average of 7 1 mEq to 19 6 mEq/l in the four children with cholera at a point along the tube about two thirds the distance (shown as zero, the reference point, Fig 1) between the ligament of Treitz and the caecum, the subsequent samples showing a further rise This point represents the sharp functional border between the jejunum and the ileum There was also a rise of pH from an average of 7 48 to 8 02 at this junction The chloride values (determine in three of four cholera patients) dropped around this region The sodium, potassium and osmolality however remained similar throughout the length of small intestine and paralleled the expected values for plasma The measured osmolalities were nearly isosmotic and close to the calculated ones

In the child with noncholera diarrhea a low pH and a low total CO_2 content were obtained throughout the small intestine (Fig 1, dotted line) The sodium level was also consistently lower while the osmolality was higher than in the cholera cases In this patient there was a difference between the osmolality directly measured by freezing point depression and the one derived from the sodium and potassium values

DISCUSSION

The luminal chemical composition of the jejunum and the ileum in secretory diarrhea due to cholera in children is similar to that in adults with cholera (11, 12) Hence the differences in stool composition between adults and children with cholera would appear to be determined by the more efficient colonic exchange of sodium for potassium in children It is of interest to note that the luminal ionic profiles of the jejunum and the ileum in those patients with cholera are similar to those in the normal fasting jejunum and ileum (6, 7, 13)

The functional jejunoileal junction in vivo is ill defined by any of the known methods such as intubation, radiology, biopsy etc Hence in various in vivo perfusion studies the selected sites have either been high up in the jejunum or deep in the ileum to avoid confusion as to the identity of the study segment The regular and sharp changes in bicarbonate and pH along the small intestine as demonstrated in this study can be used as a physiological marker to define the functional jejunoileal junction It has been shown in this study that in the secretory diarrhea of cholera in children the chemical composition across the jejunoileal region changes predictably in its direction and magnitude in keeping with the known functions of the jejunum and ileum (6, 7, 13, 15, 17, 18) These changes, are likely to hold true for normal fasting individuals as well

Unfortunately only one patient with noncholera diarrhea could be studied and no general conclusions can be confidently drawn from the findings in this patient The lower sodium and total CO_2 content in noncholera

Table 1. *Clinical and biochemical data in four children with cholera and in one with nonchole[ra] diarrhea*

				Findings on admission[a]						
								Arterial		
Patient	Age	Weight	Duration of diarrhea prior to admission (hours)	Heart rate per min	Syst B P (mmHg)	Rectal temp (C°)	Plasma sp gr	pH	P_{CO_2} (mEq/l)	Base excess (mEq/l)
H23	5 0	11 92	10	154	80	39 4	1 033	7 116	7 8	−21 8
H59	4 3	9 19	6	160	70	39 4	1 033	7 216	12 0	−15 0
H63	5 3	9 46	15	180	44	37 8	1 035	7 226	14 5	−12 8
H64	5 0	10 98	9	120	56	37 6	1 032	7 245	10 4	−15 8
H65	5 5	11 63	4	158	60	38 0	1 029	7 305	17 5	− 7 5

[a] Patient H23 and H63 were in stupor, Patient H59 was conscious and irritable, and Patients H64 and H65 were conscio[us] but lethargic All had moderate to marked loss of skin turgor First 4 children had cholera, the last one had acute nonchole[ra] diarrhea

with noncholera diarrhea. The results also define the functional jejunoileal junction

MATERIALS AND METHODS

Five children with moderate to severe dehydration due to acute watery diarrhea of less than 24 hours duration and who had no antibiotics prior to admission were studied During the entire study period rehydration and correction of acidosis were carried out with appropriate I V fluids according to a scheme described earlier (11) and nothing was given orally Blood acid–base parameters measured at the beginning and at the end of sampling were within the normal range The children were intubated soon after admission with a soft fine (I D =0 6 mm) biluminal P V C tube with an inflatable mercury balloon at the end of radioopaque tube the utmost care was taken to prevent traction on the tube during the passage by peristalsis and the position of the tube was checked by fluoroscopy Paired samples at 10 cm intervals were collected for microbial culture and biochemical analysis until the balloon reached the caecum or ascending colon the final position being checked by fluoroscopy after injecting Hypaque® (Winthrop) The tube dead space was emptied before obtaining each sample Samples were analysed immediately for pH, total CO_2 and osmolality and aliquots were frozen at −40°C for electrolytes Plasma specific gravity was measured by refractometry (TS Meter American Optical Company), arterial blood acid–base parameters on an Astrup Microtonometer, plasma and stool sodium and potassium on a Patwin internal lithium standard flame photometer, osmolality by freezing point depression (Fiske Osmometer) and pH on a Radiometer pH meter with a thermostated electrode All analyses were performed in duplicate Microbiological methods and their results in these subjects have already been described (8 14) This study forms a part of a series of studies aimed at evolving rational therapy for cholera, initiated at a time when child mortality from this desease was high Informed consent was obtained from the parents The study was approved by the ethical committees of the Institutes involved

RESULTS

Clinical and biochemical investigations on these five children revealed a moderate to severe degree of dehydration on admission with base deficit acidosis The duration of watery diarrhea prior to admission varied from 4 to 15 hours (Table 1) Small intestinal and stool samples from the four children with cholera (H23, H59, H63, H64) showed *Vibrio cholerae El Tor* with counts ranging from 10^4 to 10^6 per ml (8) In addition, components of normal flora such as *E coli* streptococci, anaerobic lactobacilli and fungi were present throughout the length of the small intestine The child with noncholera diarrhea (H65) had *E coli* with counts from 10^4 to 10^6 per ml throughout the small intestine which rose to 10^9 per ml in the stool The *E coli* strains from the stool were enterotoxigenic in the rabbit ileal loop (14) Stool culture, however, also grew Shigella boydii (10^7 per ml)

The stool composition (Table 1) in the four cholera patients was similar to that previously reported for cholera in children (11, 12) The stools were alkaline, nearly isosmotic, and there was little discrepancy between the meas-

Stool composition				
Na^+ (mEq/l)	K^+ (mEq/l)	Total CO_2 (mEq/l)	Osm (mOsm/kg)	pH
91 2	29 6	37 8	–	7 95
39 0	99 9	29 4	290	7 44
105 5	37 7	34 7	275	7 41
101 5	49 7	49 2	303	7 45
65 3	31 4	15 0	–	–

ured osmolality (measured in three of the four children with cholera) and the derived one i e twice the sum of the sodium and potassium values However, the stool sodium as well as the sum of the sodium plus potassium values were much lower in the only child with non cholera diarrhea (H65) which is in keeping with previous studies on infantile diarrhea (9, 11 12)

Chemical composition of small intestinal fluid Total CO_2 content rose abruptly from an average of 7 1 mEq to 19 6 mEq/l in the four children with cholera at a point along the tube *about two thirds the distance (shown as zero* the reference point, Fig 1) between the liga ment of Treitz and the caecum, the subse quent samples showing a further rise This point represents the sharp functional border between the jejunum and the ileum There was also a rise of pH from an average of 7 48 to 8 02 at this junction The chloride values (determine in three of four cholera patients) dropped around this region The sodium, potassium and osmolality however remained similar throughout the length of small intestine and paralleled the expected values for plasma The measured osmolalities were nearly isosmotic and close to the calculated ones

In the child with noncholera diarrhea a low pH and a low total CO_2 content were obtained throughout the small intestine (Fig 1, dotted line) The sodium level was also consistently lower while the osmolality was higher than in the cholera cases In this patient there was a difference between the osmolality directly measured by freezing point depression and the one derived from the sodium and potassium values

DISCUSSION

The luminal chemical composition of the jejunum and the ileum in secretory diarrhea due to cholera in children is similar to that in adults with cholera (11, 12) Hence the differences in stool composition between adults and children with cholera would appear to be determined by the more efficient colonic exchange of sodium for potassium in children It is of interest to note that the luminal ionic profiles of the jejunum and the ileum in those patients with cholera are similar to those in the normal fasting jejunum and ileum (6, 7, 13)

The functional jejunoileal junction in vivo is ill defined by any of the known methods such as intubation, radiology, biopsy etc Hence in various in vivo perfusion studies the selected sites have either been high up in the jejunum or deep in the ileum to avoid confusion as to the identity of the study segment The regular and sharp changes in bicarbonate and pH along the small intestine as demonstrated in this study can be used as a physiological marker to define the functional jejunoileal junction It has been shown in this study that in the secretory diarrhea of cholera in children the chemical composition across the jejunoileal region changes predictably in its direction and *magnitude in keeping with the known* functions of the jejunum and ileum (6, 7, 13, *15, 17, 18) These changes, are likely to hold* true for normal fasting individuals as well

Unfortunately only one patient with noncholera diarrhea could be studied and no general conclusions can be confidently drawn from the findings in this patient The lower sodium and total CO_2 content in noncholera

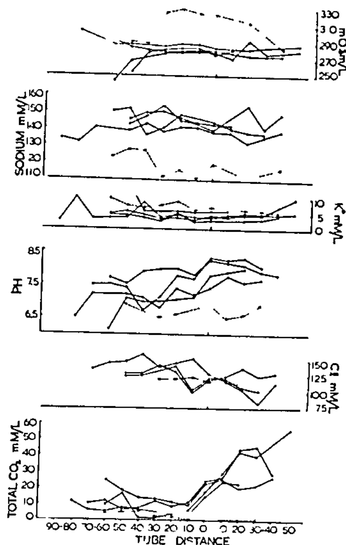


Fig 1 Intestinal fluid composition at 10 cm intervals are plotted In each patient the point of sharp rise in total CO_2 content (approximately two thirds down the tube distance between the ligament of Treitz and caecum) in 4 children with cholera is shown as the zero reference point and values from proximal and distal samples have been plotted accordingly identifying them by a negative or a positive number corresponding to the tube distance (cm) from this reference point Hence the most proximal one or two samples are duodenal The data from the child with non cholera diarrhea are shown by dotted lines the zero reference point however corresponds to two thirds the tube distance between ligament of Treitz and caecum there being no rise in total CO_2 content

diarrhea stools of infants and children are believed to largely reflect the composition in the proximal jejunum Nonabsorbable solutes such as organic acids from bacterial metabolism (16), unabsorbed sugars due to disaccharidase deficiency (4), fatty acids etc lead to the osmotic accumulation of fluid in the lumen, thus lowering the sodium concentration although the measured osmolality remains high The organic acids of bacterial origin displace bicarbonate ions, leading to a low total CO_2 and a low pH All these chemical events high up in the jejunum are reflected in the stool composition in this patient with non cholera diarrhea More patients with infantile diarrhea due to other causes should be studied to confirm these impressions These findings if confirmed, are relevant to the optimum composition of oral rehydration fluids in infantile diarrhea (4, 5) as well as for children with cholera since a higher sodium concentration of oral rehydration fluids may carry a risk of producing a salt overload (5) in the former

ACKNOWLEDGEMENT

I acknowledge help from Drs C N Das J Brayton N F Pierce K N Jalan and technical assistance of Mr Samir Biswas

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Submitted April 24, 1979
Accepted Aug 29, 1979

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Acta Pædiatr Scand 69 231–233, 1980

LIPIDS AND LIPOPROTEINS IN 350 DANISH SCHOOLCHILDREN, AGES 7 TO 18 years

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ABSTRACT Ibsen, K K , Lous, P & Andersen, G E (University Clinic of Paediatrics, Childrer ... the Department of Clinical Chemistry, Bispebjerg Hospital and the ... and lipo ... 1980.— ... 7 to 18 years ... population ... icantly with ... adolescence ... American children, suggesting that Danish children may be more prone to develop coronary heart disease in adult life

KEY WORDS Cholesterol, triglyceride, lipoprotein, childhood, adolescence

Hyperlipoproteinemia is one of the best known risk factors for developing coronary heart disease (CHD) In a prospective study (1) it has recently been shown that the number of non fatal cardiac infarctions can be reduced by lowering an elevated serum cholesterol level The study was made in men above 30 years of age and it is likely that the results would have been even better, had reduction of serum cholesterol been initiated before age 30 It is well known that certain forms of hyperlipoproteinemia can be diagnosed in childhood and one form, viz familial hyper cholesterolemia (FH), already at birth (2) It has become clear within recent years that the measurement of the lipoprotein subfractions (VLDL, LDL and HDL) offers a more specific assessment of the individuals with an in creased risk to CHD (elevated LDL and/or decreased HDL) or a reduced risk to CHD (elevated HDL and/or decreased LDL) (3, 4, 5, 6 7)

The present study was undertaken to provide the Scandinavian pediatrician with percentile values for lipids and lipoproteins in a reference group of schoolchildren in order to make possible an early detection of those putatively at increased and decreased risk to CHD during adult life

MATERIAL

185 white boys and 165 white girls were selected at random from families in which the parents had earlier been selected at random from the public registry office in East Copenhagen for participation in a coronary risk factor study Informed parental consent was obtained and a venous blood sample was drawn at school be tween 8 and 11 a m without prior fasting The lipid and lipoprotein determinations were begun the same day as the blood was drawn

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Abbreviations

CHD = Coronary Heart Disease
FH = Familial Hypercholesterolemia
VLDL-C = Very Low Density Lipoprotein Cholesterol
LDL-C = Low Density Lipoprotein Cholesterol
HDL-C = High Density Lipoprotein Dholesterol
T-C = Total Cholesterol
TG = Triglyceride

Table 1 *Percentile values for serum lipids and lipoproteins in 7–18 year old Danish children*

n	Boys	Girls	Ages (years)	Total cholesterol (mmol/l)					VLDL-cholesterol (mmol/l)				
				5	10	50	90	95	5	10	50	90	95
82	57	25	7–10	3 45	3 64	4 48	5 46	5 74	0 05	0 08	0 18	0 43	0 52
114	55	59	11–13	3 32	3 59	4 44	5 70	6 24	0 08	0 11	0 24	0 49	0 61
60	36	44	14–15	2 86	3 40	4 49	5 59	5 87	0 11	0 12	0 24	0 44	0 59
74	37	37	16–18	3 50	3 60	4 58	5 45	6 14	0 08	0 12	0 28	0 49	0 64
350	185	165	7–18	3 37	3 61	4 47	5 48	5 96	0 08	0 10	0 24	0 48	0 59

METHODS

The methods for measuring cholesterol and triglyceride are the ones already described (2) VLDL C was measured manually in duplicate after ultracentrifugation in a 40 3 rotor Beckman Type L ultracentrifuge at 10°C at 40000 r p m for 20 hours and after tube slicing HDL C was measured enzymatically on a Greiner Selective Analyzer II as described by Borner & Klose (8) after precipitation of VLDL LDL with dextran-sulphate-$MgCl_2$ (9) The day to-day variation was estimated by including a sample of frozen pool serum which was thawed precipitated and included in each run The coefficient of variation for HDL C values in 24 pool sera was found to be 5 3%

Non parametric statistics as described by Siegel (10) were used

RESULTS

Percentile values for serum lipids and lipoproteins are given in Table 1 The lipid and lipoprotein values were compared in boys and girls belonging to the age groups given in Table 1 No sex differences were found and therefore percentile values for boys and girls were presented together For the convenience of the reader the percentile values for all age groups are summarized at the foot of Table 1 As can be seen, only VLDL C and TG concentrations showed a significant rise with age ($p<0\ 001$)

DISCUSSION

Several studies from the United States (3 11 12, 13) and Scandinavia (14 15 16) have included reference values for T C and TG in schoolchildren Few studies, however have also included reference values for lipoproteins Ultracentrifugation alone or in combination with polyanion precipitation of lipoproteins as in the present study was used in the four American studies (3, 11, 12, 13)

In two of these (12 3) the median value for VLDL C was 0 18 and 0 31 mmol/l resp which is close to 0 24 in the present study The 95 percentile values for VLDL C were found to be a little higher (0 64 and 0 73 mmol/l resp) than our value of 0 59 mmol/l

In the four American studies median values for LDL C were 2 23–2 86 mmol/l which is lower than our value of 2 88 mmol/l The American 95 percentile values of 3 35–4 16 mmol/l were also lower than our value of 4 18 mmol/l

The American median values for HDL C of 1 35–1 72 mmol/l and 95 percentile values of 1 86–2 60 mmol/l were higher than those found in the present study In spite of methodological differences between and within the above mentioned studies and the present one there seems to be a tendency towards higher LDL C and lower HDL C values in Danish schoolchildren who may be more prone to developing CHD than are American schoolchildren Whether these differences are caused by ethnic dietary or still other factors is not known at present

The increase in TG and VLDL C values with age in boys in the present study confirms the findings by Srinivasan et al (12) and Morrison et al (13) They also found a trend towards decreased LDL C values with age in white girls which we could not confirm since in the present study LDL C values remained constant during adolescence

HDL C values remained unchanged during adolescence both in the two studies mentioned above (12 13) and in the present study

	L cholesterol (mmol/l)				HDL-cholesterol (mmol/l)					Triglyceride (mmol/l)				
	10	50	90	95	5	10	50	90	95	5	10	50	90	95
6	2 24	2 91	3 85	4 03	0 99	1 08	1 32	1 66	1 82	0 45	0 49	0 74	1 45	1 64
1	2 16	2 82	3 87	4 49	0 92	0 96	1 29	1 74	1 87	0 44	0 55	0 86	1 58	2 46
5	1 84	2 91	3 99	4 05	0 90	1 00	1 28	1 55	1 67	0 48	0 60	0 98	1 69	1 93
1	2 23	2 90	3 79	4 26	0 82	0 91	1 23	1 61	1 69	0 56	0 67	1 02	1 79	1 94
1	2 13	2 88	3 88	4 18	0 91	0 97	1 29	1 64	1 77	0 46	0 57	0 88	1 60	1 86

ACKNOWLEDGEMENT

This study was supported by grants from the Danish Heart Foundation and the Dagmar Marshall Fund

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Submitted July 24 1979
Accepted Sept 7 1979

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Table 1. *Percentile values for serum lipids and lipoproteins in 7–18-year-old Danish children*

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80	36	44	14–15	2 86	3 40	4 49	5 59	5 87	0 11	0 12	0 24	0 44	0 5[illegible]
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350	185	165	7–18	3 37	3 61	4 47	5 48	5 96	0 08	0 10	0 24	0 48	0 5[illegible]

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The increase in TG and VLDL-C values with age in boys in the present study confirms the findings by Srinivasan et al (12) and Morrison et al (13) They also found a trend towards decreased LDL C values with age in white girls, which we could not confirm, since in the present study LDL-C values remained constant during adolescence

HDL-C values remained unchanged during adolescence both in the two studies mentioned above (12, 13) and in the present study

Acta Pædiatr Scand 69 235–238, 1980

TRANSIENT HYPERPHOSPHATASEMIA OF INFANCY

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ABSTRACT. Nathan, E. (Department of Paediatrics, Kolding Hospital, Kolding, Denmark). Transient Hyperphosphatasemia of Infancy. Acta Paediatr Scand, 69.235, 1980.—Striking transient increases in serum alkaline phosphatase in 6 infants are reported. Isoenzymes were studied in 3 infants and increased activity was found in bone as well as in liver fractions. Repeated serum determinations demonstrated a duration of about 11 weeks with a peak serum value in the 6th week The etiology is unknown but an infectious cause is discussed. The condition may be rather frequent but gives no obvious symptoms It is important to know this condition to avoid unnecessary diagnostic procedures

KEY WORDS Alkaline phosphatase, transient hyperphosphatasemia

In 1954 Bach (2) described 3 cases of a striking transient increase in the serum alkaline phosphatase (AP) among apparently healthy infants Since then the phenomenon has been described among healthy infants (1) as well as among infants with various diseases (9) Posen et al (9) have proposed to call the syndrome transient hyperphosphatasemia of infancy

Until now 28 cases have been reported. No etiologic agent has been found In all the described cases the AP was normalized within less than 12 weeks Repeated enzyme estimations during these weeks have not previously been reported In this report of 6 infants with transient hyperphosphatasemia, isoenzyme fractionation has been performed in 3 of them as well

METHODS

The AP was measured in cases 1 and 2 as described by Bessey et al (3) and in cases 3 to 6 as recommended



alanin inhibition was not carried out In case 3 fractionation was performed by a commercial laboratory (Medicinsk Laboratorium, Copenhagen) combining heat inactivation (56°C for 6 minutes) and agargel electrophoresis (10)

CASE REPORTS

Case 1

A boy with a birth weight of 3 25 kg At the age of 16 months he was admitted to the Department of Paediatrics, Kolding Hospital He appeared pseudoretarded due to lack of stimulation and had divergent strabismus He showed no clinical signs of rachitis He had a mild hyperchromic anemia and the AP was elevated (Fig 1) The epiphysis of the wrist appeared normal on X ray The serum calcium and phosphate were normal He was suspected of having a slight rachitis and was treated with calcium and supplementary vitamin D, the effect being controlled weekly by the AP When the result of the 3rd sample (Fig 1) appeared, a laboratory failure was suspected to be present Therefore, additional samples were analysed on the next day and then again 4 days later The AP increased progressively with a maximum of 3 930 U/l, more than 20 times the highest normal upper limit for infants As demonstrated in the figure, the AP then decreased Isoenzymes were measured when the AP was at the maximum, and increased levels of intestinal, bone, and liver isoenzymes were demonstrated

During the 5½ weeks when the boy was in hospital his temperature was normal and he did not show any signs of disease His motor development improved He was readmitted twice, after 3 weeks and 4 years, respectively, and on both occasions the AP was normal

During the transient hyperphosphatasemia, white blood count, sedimentation rate, aspartate-aminotransferase, lactate dehydrogenase, calcium and phosphate in serum were found normal Microscopy of urine was normal The titres of respiratory syncytial, of influenza A and B, of cytomegalo virus, and of toxoplasma were normal

Case 2

A girl born 3 weeks before term, birth weight 2 40 kg At the age of 12 months she was admitted to the Department of Paediatrics, Kolding Hospital, because of re-

Table 1. *Transient hyperphosphatasemia of infancy as reported in 6 publications*

	n	Sex m/f	Age (months)	Presentation	Duration (weeks)	Fractionation
Bach (2)	3 (7.5% of the normal controls)	–	2–18	"Normal control	6–8	–
Asanti et al. (1)	(1.1%–3.5% of the normal controls)	–	2–15	Normal control"	6–12	–
Geudeke (5)	1	0/1	24	–	–	Bone and liver
Posen et al. (9)	5	2/3	5–18	Various clinical features	5–7	Bone
Wieme (11)	11	6/5	9–34	Various clinical features	5–10	Bone and liver
This paper	6	3/3	8–17	Various clinical features	max. 11	Bone and liver

DISCUSSION

The 17 cases described by Geudeke (5), Posen et al. (9), and Wieme (11) and the 6 cases in this report presented different reasons to estimate the AP. None of the infants, however, showed clinical evidence of hepatic or bone diseases and no symptoms or treatments were common for all the children.

11 of the previously reported cases of transient hyperphosphatasemia were found among apparently healthy infants (Table 1). Bach (2) repeatedly examined 40 children without rachitis and thereby found 3 infants with a pronounced increase of the AP. Asanti et al. (1) found 8 cases. They examined three age groups and found the condition in 1.2% in the group 2–4 months, 1.1% in the group 4–7 months and 3.5% in the group 12–15 months. The incidence of the condition cannot be stated from these figures. It may be high owing to the fact that most of the cases are probably not diagnosed as the condition gives no obvious symptoms. The 6 cases in this report appeared during a period of 4 years.

The condition has been described only among infants of 2–34 months with no sex difference.

The course of enzyme activity in transient hyperphosphatasemia has not previously been demonstrated. In case 1 the AP was analysed repeatedly. Within a rather short period, an almost exponential increase ... appeared and exceptionally high values were reached, followed by a rapid fall. But not until several weeks was the enzyme within normal range. If the decreasing values of cases 1, 2, 4, and 6 are superimposed upon each other, all the values of the cases mentioned will form the curve shown in Fig. 1. The values of the remaining 2 cases coincide with this curve. It is probable that Fig. 1 shows the real course of the changing enzyme activity of transient hyperphosphatasemia of infancy. It demonstrates a duration of about 11 weeks with a peak serum value in the 6th week. 4 of the 6 cases in this report were re-examined from 3 months to 4 years later. The AP was normal and the patients were found healthy.

Fractionation of the AP has been carried out in some cases of transient hyperphosphatasemia. By heat inactivation and in some cases also by L-phenylalanin inhibition and electrophoresis, Posen et al. (9) found that the enzymes were originating only from bone. Wieme (11) found a double origin for the increase—from bone and from liver—although he found an unexplained slight difference in mobility of liver fraction in agargel as compared with liver-derived AP of adult sera. Also Geudeke (5) found both bone and liver isoenzyme present. In cases 1, 3, and 6 of this report, increases in both liver and bone fractions of the enzyme were found. The increased activity in the intestinal isoenzyme found in

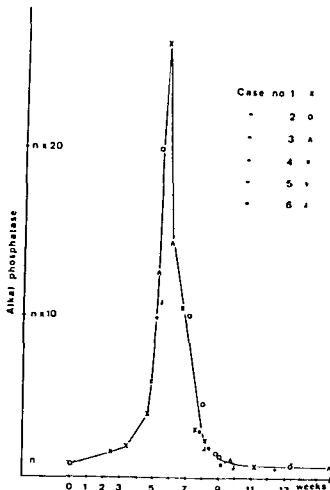


Fig. 1 All the AP values measured in the 6 cases during the transient hyperphosphatasemia Week 0 indicates the last normal value prior to the syndrome n = the highest normal activity for the AP in childhood according to the laboratory In cases 1 and 2 the AP was measured as described by Bessey et al (3) $n = 150$ U/l and in cases 3–6 as recommended by the Scand Soc (4) $n = 1\,000$ U/l

current bronchitis The AP was normal (Fig 1) She had been given vitamin D 600 I U per day instead of 1 200 I.U as we recommend for prematures 5 weeks later she was examined in the out-patient clinic and was found in good health The AP was now 2 950 U/l, i e about 20 times the normal upper limit Serum calcium, phosphate, lactate dehydrogenase, aspartat-aminotransferase, hemoglobin, white blood count, and urine microscopy were all normal X-ray of the epiphysis showed no rachitis 3 weeks later she was readmitted with bronchopneumonia and a temperature of 38 2°C Serum-transaminases, serum calcium, and phosphate levels were still normal The adenovirus titre increased to 1 128 The titres of cytomegalo-, of respiratory-syncytial-, of influenza A and B virus, and of toxoplasma were normal No hepatitis associated antigen was found 3½ years later she was readmitted due to recurrent bronchitis 2 months later she was examined in the out-patient clinic and was in good health The AP was not analysed on these occasions

Case 3

A girl with birth weight 3 25 kg Due to two attacks of febrile convulsions the girl was treated prophylactically with phenobarbital.

At the age of 17 months she was admitted to the Neurological Department, Vejle Hospital, with her 3rd attack of febrile convulsions The AP was 12555 U/l (Fig 1). Serum calcium and phosphate were normal No explanation of the fever was found She was controlled one week later in the outpatient clinic Her temperature was 40 5°C and a tonsillitis was treated with penicillin The AP was 14550 U/l Fractionation of the AP showed elevation of both liver and bone isoenzymes 4 weeks later she was examined in the out patient clinic of the Paediatric Department, Kolding Hospital Serum aspartate amino-transferase was normal. The epiphysis of the wrist was normal on X-ray. At a follow-up 3 years later the girl was in good health and the AP was normal

Case 4

At the age of 13 months this girl was admitted to Brø-strup Hospital because of febrile convulsions She w found to have an otitis with spontaneous perforatio Her temperature was 39 7°C, declining to normal dur

A slight lymphocytosis was found

Case 5

exanthema subitum The AP was 10 000 U/l Serum-calcium, -phosphate, -transaminase, and lactate dehydrogenase were normal Leukocyte count was 16 9×10⁹ with a normal distribution Unfortunately, the high AP

AP was normal (Fig 1) Another control 4 months late showed the same result

Case 6

A premature boy weighing 0 85 kg at birth The neonatal period was uneventful At the age of 8 months he was examined in the out patient clinic Until then, vitamin D 1 200 I U daily and iron supplement was given The AP was 11 900 U/l (Fig 1) The boy was in good health Serum calcium and phosphate were normal A few weeks later the serum aspartate aminotransferase was normal Toxoplasma titre and cytomegalovirus titre were normal X-ray of the wrist showed retarded bone age but no rachitis 3 weeks later the AP was 1 955 U/l (Fig 1), and fractionation of the AP showed increased levels of intestinal, bone, and liver isoenzymes Controls 4½ weeks and 2½ months later showed that he was in good health and had normal AP

GROWTH PATTERNS OF CARDIAC STRUCTURES AND CHANGES IN SYSTOLIC TIME INTERVALS IN THE NEWBORN AND INFANT

A Longitudinal Echocardiographic Study

I OBERHANSLI G BRANDON G LACOURT and B FRIEDLI

From the Division of Cardiology and Neonatology Department of Pediatrics and Genetics University of Geneva Switzerland

ABSTRACT Oberhansli, I, Brandon, G, Lacourt, G and Friedli, B (Department of Pediatrics and Genetics, University Hospital, Geneva, Switzerland) Growth patterns of cardiac structures and changes in systolic time intervals in the newborn and *infant* Acta Paediatr Scand, 69 239, 1980.—A longitudinal study was undertaken in 21 newborns to determine cardiac growth pattern by echocardiography over the course of the first year of life Most cardiac structures increased in size as a linear function of age and weight, however, the right ventricular end-diastolic diameter remained unchanged so that the RV/LV ratio decreased as a parabolic functi [illegible] Left and right ventricular systolic time intervals (RVSTI, LVSTI) after birth were a [illegible] ventricular ejecti [illegible] ly remained at a [illegible] ventricular systo [illegible] during the first d [illegible] on the 6th day of life) Constant values of [illegible] onwards The decrease in RVPEP/RVET in the first days of life followed a parabolic function reflecting the physiological decrease of pulmonary vascular resistance after birth

KEY WORDS Healthy newborns longitudinal study, cardiac diameters, systolic time intervals

M mode echocardiography is now routinely used for evaluation of normal and diseased cardiac structures in children and adults (5 8 15 24 27 32 34) Normal growth patterns and growth related changes of intracardiac structures dimensions and function in relation to either weight or body area have been published from cross sectional studies

A longitudinal study which follows the growth pattern from birth to one year of age in normal newborns has not been previously reported

We therefore studied a cohort of normal newborn babies sequentially by M mode echocardiography during the first year of life The results were correlated with age weight and

METHOD

Serial echocardiograms were obtained in 21 newborn babies (12 males 9 females) within 24 hours after birth and subsequently at 3 days 6 days 30 days and 60 days of age Further echocardiographic data were collected between 6 and 12 months in 15 of these babies These 21 randomly selected newborns had a gestational age of 37–41 weeks Fifteen of them were normally delivered and had Apgar scores of 8–10 in 5 min The remaining 6 were delivered by caesarian section for maternal indications None of them had any postnatal problems

General cardiac examination and follow up studies showed the absence of heart disease in the group except in one female baby who had a small VSD She remained asymptomatic with normal ECG and chest X ray All echocardiographic examinations were performed after feeding They were taken in the supine position no sedation was used A Picker 80 C M mode echocardiograph with a fiberoptic strip chart recorder or an IREX II echocardiograph and a 5 or 3 5 MHZ unfocused transducer were used

cases 1 and 6 was probably due to a misinterpretation of the overlapping part of the overwhelming bone fraction L phenylalanin inhibition was not carried out

It is unknown whether the increased activity of the AP is caused by a greater quantity of the enzyme in the blood or by an activation of a normal amount of the enzyme The increased activity of different isoenzymes suggests the latter possibility It is known that Riley virus in mice can increase certain enzymes (8) The transient course in all cases described suggests that an infectious agent is responsible for the condition In case 2 an increasing adenovirus titre was found The patient had bronchitis, and the finding may be incidental Adenovirus titre was not tested in the other patients

Transient hyperphosphatasemia can easily be misinterpretated as a laboratory failure or, as in case 1, as rachitis Though the condition does not seem to give any symptoms, it is important to know of its existence in order to avoid unnecessary diagnostic procedures when looking for other diseases associated with increased serum alkaline phosphatase activity

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Submitted June 12 1979
Accepted Sept 18 1979

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Acta Pædiatr Scand 69 239–247, 1980

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A longitudinal study which follows the growth pattern from birth to one year of age in normal newborns has not been previously reported

We therefore studied a cohort of normal newborn babies sequentially by M mode echocardiography during the first year of life The results were correlated with age, weight and length

METHOD

Serial echocardiograms were obtained in 21 newborn babies (12 males 9 females) within 24 hours after birth and subsequently at 3 days 6 days 30 days and 60 days of age Further echocardiographic data were collected between 6 and 12 months in 15 of these babies These 21 randomly selected newborns had a gestational age of 37–41 weeks Fifteen of them were normally delivered and had Apgar scores of 8–10 in 5 min The remaining 6 were delivered by caesarian section for maternal indications None of them had any postnatal problems

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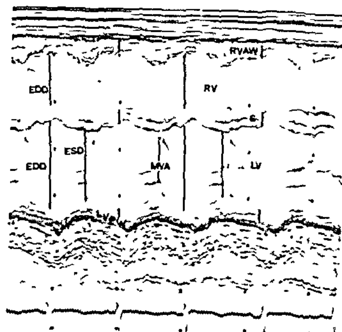


Fig. 1. Echocardiogram showing the right and left ventricular cavity. The vertical lines indicate structural measurements. *RVAW*, Right ventricular anterior wall, *RV*, right ventricle, *S*, interventricular septum, *LV*, left ventricle, *LVPW*, left ventricular posterior wall, *EDD*, end diastolic diameter, *ESD*, end systolic diameter, *MVA*, mitral valve amplitude.

The echocardiograms were obtained from the standard precordial position (5, 7, 11, 21, 22, 33, 36) previously described.

The ECG lead which most clearly demonstrated early ventricular depolarization was chosen for timing the onset of electrical systole.

The following cardiac dimensions were measured: right ventricular anterior wall thickness (RVAW), right ventricular and left ventricular end diastolic diameter (RVD and LVEDD), interventricular septal thickness (IVS) and left ventricular posterior wall thickness at the onset of the R wave using the leading edge method except for RVAW. RVAW was measured from the inner surface of the chest wall echo to the right ventricular endocardial surface echo.

Left ventricular end systolic dimension (LVESD) was taken at the peak of the upward motion of the posterior left ventricular endocardial echo to the left side of the IVS (Fig. 1).

Measurements of the aortic root and the right ventricular outflow tract (RVOT) were obtained using the leading edge method (outer/inner echos for the aortic root and right ventricular endocardial echo to the inside of the anterior aortic wall echo for RVOT) on a mitral aortic sweep at end-diastole (i.e. at the onset of the QRS complex) (Fig. 2*a*).

Left atrial dimension was taken at the point of most anterior position of the aortic root using the leading edge method, i.e. from the inside of the posterior aortic wall echo to the inside of the left atrial posterior wall echo.

Pulmonary artery root dimension was measured whenever the anterior wall echo moved parallel to the posterior pulmonary wall echo at any time of the cardiac cycle, preferably at the onset of the QRS complex.

Maximal aortic and pulmonary valve separation was measured in systole, immediately after the valve opening. Mitral and tricuspid valve depths were measured from the outside of the chest wall echo to the D point of the mitral or tricuspid valve echo, respectively.

The anterior mitral and tricuspid leaflet excursion was taken from the D point of the concerned valve to the E point at maximal excursion (5).

The E–F slope, representing the closing velocity of the anterior leaflets at the A–V valves, was determined by the use of the triangle method (22).

Three to five well defined complexes were selected for measurements and the obtained values were then averaged.

Each measurement was analysed with respect to age

regression line was a first or second degree polynomial function of the age, the weight or the height. Statistical analysis was performed using the MUMPS system (2, 23, 34).

Right and left ventricular systolic time intervals (STI) were studied as indicated previously (16, 17). Special care

Table 1. *Cardiac dimensions (mean ± 1 S.D.)*

Age	Weight (kg)	Length (mm)	LV/EDD (mm)	LV/ESD (mm)	LVPW (mm)	SEPTUM (mm)	RV/EDD (mm)	RVOT (mm)	RVAW (mm)
0–24 hours	3 283±0 478	49 85±2 0	17 3±1 6	11 6±1 3	3 1±0 2	3 2±0 2	10 7±2 7	14 6±1 4	2 6±0 3
3 days	3 215±0 481	49 90±1 97	17 0±1 4	11 5±1 1	3 3±0 3	3 3±0 3	9 8±1 1	13 8±1 8	2 7± 02
6 days	3 194±0 466	50 00±1 97	17 4±1 3	11 7±0 9	3 4±0 3	3 5±0 3	9 7±1 3	13 8±1 6	2 8±0 2
1 month	3 714±0 446	–	19 5±1 8	13 1±1 3	3 7±0 4	3 7±0 4	9 5±1 3	14 2±1 4	2 9±0 2
2 months	4 618±0 423	–	22 0±2 0	14 7±1 8	4 0±0 5	4 1±0 5	10 9±1 2	14 8±1 5	2 9±0 2
6–12 months	9 149±1 268	72 35±3 36	25 4±3 0	16 0±2 3	4 9±0 6	5 0±0 6	10 2±1 7	16 0±3 1	3 1±0 2

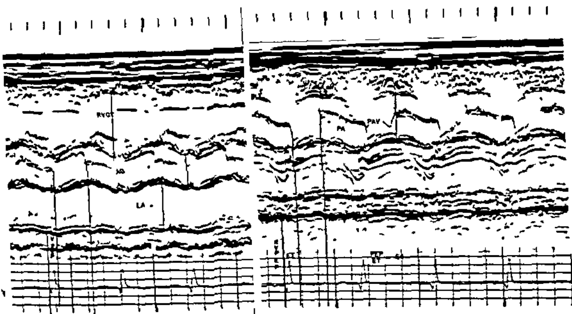


Fig 2a b Pulmonic and aortic valve echogram from a normal term neonate in order to demonstrate measurements of STI The time lines are at 100 msec *RVOT* Right ventricular outflow tract *LA* left atrium *Ao* aorta *LVPEP* left ventricular pre ejection period *LVET* left ventricular ejection time *PAV* pulmonary artery valve, *PA* pulmonary artery *RVPEP* right ventricular pre-ejection period *RVET*, right ventricular ejection time

was taken to obtain complete echos of the aortic valve and the pulmonary artery valve so that the systolic time intervals could be determined exactly In the case of the aortic valve this was always possible In the pulmonary valve echogram however it was often only possible to visualise the posterior valve leaflet In these cases the following definitions were used start of ejection time=meeting point of the thin and the tick valvular echo end of ejection time=either the meeting point between the posterior and the anterior thin valvular echo or the meeting point between the posterior thin echo and the thicker diastolic valvular echo (Fig 2a b)

The echocardiographic tracings were obtained with a paper speed of 100 mm/sec time lines of 40 msec facilitated the measurements of the STI to the closest 5 msec

Pre-ejection period/ejection time (PEP/ET) ratios were calculated for both ventricles and correlated with the age of the infants

RESULTS

The results of the serial examinations in the 21 newborn babies are given in Table 1 For each diameter and each age group, the mean

MV/Ampl (mm)	MV/E-F slope (mm/sec)	TV/Ampl (mm)	TV/E-F slope (mm/sec)	TV/MV TV/MV	Ao/Root (mm)	LA (mm)	LA/Ao	PA/Valve (mm)
7 4±0 9	51 0±14 1	8 6±1 1	47 0±12 5	1 2±0 1	9 8±1 0	11 9±1 8	1 2±0 2	8 3±1 1
7 6+1 1	53 2±11 8	8 2±1 0	42 5±15 8	1 1±0 1	10 0±0 9	11 6±1 2	1 2±0 1	8 5±0 8
7 9±1 2	51 5±10 5	8 2±1 2	45 3±13 2	1 0±0 1	10 3±0 8	11 8±0 9	1 2±0 1	8.5±0 8
9 0±1 2	69 8±13 4	9 1±1 3	52 6±14 1	1 0±0 1	11 2±0 9	12 9±1 2	1 2±0 1	8 9±0 9
9 3±1 4	65 8±14 5	8 8±1 2	65 8±17 7	1 0±0 1	11 8±1 2	14 0±1 3	1 2±0 1	9 7±1 1
11 5±1 9	76 3±15 6	11 2±1.5	65 5±14.5	1 0±0 04	14 0±1 5	16 1±2 1	1 2±0 1	11 4±0 7

Table 2. *Cardiac dimensions as a function of weight (kg)*

SEE=standard error of the estimate, r=correlation coefficient, p=probability, y=ordinate (dimension in mm) x=abscissa (weight in kg, non logarithmic)

		SEE	r	p
End-diastolic dimension of LV	$y=1\ 38x+13\ 42$	1 91	0 83	<0 001
End systolic dimension of LV	$y=0\ 77x+\ 9\ 55$	1 49	0 72	<0 001
Left ventricular posterior wall	$y=0\ 26x+\ 2\ 54$	0 38	0 81	<0 001
Interventricular septum	$y=0\ 26x+\ 2\ 59$	0 44	0 77	<0 001
Aortic root diameter (Ao)	$y=0\ 66x+\ 8\ 20$	1 04	0 79	<0 001
Aortic valve separation	$y=0\ 54x+\ 5\ 99$	0 72	0 83	<0 001
Left atrial diameter (LA)	$y=0\ 69x+\ 9\ 88$	1 49	0 68	<0 001
[illegible]	$y=1\ 36x+29\ 17$	2 40	0 75	<0 001
[illegible]	$y=0\ 63x+\ 5\ 91$	1 28	0 70	<0 001
[illegible]	$y=1\ 10x+19\ 77$	1 87	0 76	<0 001
[illegible]	$y=0\ 55x+\ 9\ 54$	1 01	0 75	<0 001
[illegible]	$y=0\ 47x+\ 7\ 02$	0 92	0 73	<0 001

value ±1 S D was calculated Equations relating dimensions to weight are given in Table 2. The increase in dimensions of LV, LA, Ao, as well as LVPW, IVS and RVAW with body weight are best described as a linear regression

Because some measurements may vary as a function of age rather than weight, especially those related to the changes in circulation after birth, echocardiographic dimensions were correlated with age The equations of the corresponding regression lines (dimension versus log of age) are presented in Table 3 for each diameter These essentially show that LA, LV, Ao and the ventricular walls grow linearly with the natural log of age whereas the RV dimension does not change (Fig 3) Thus RV/LV decreases with age, this change is best demonstrated using the ratio tricuspid valve/mitral valve amplitude (Fig 4) and fits a parabolic function The LA/Ao ratios could also be characterized by a parabolic function during the first six days of life From the 6th day on they became constant For the total group a paired t-test of LA/Ao ratios between the 1st and 3rd, and 3rd and 6th day was, however, not significant

Left and right ventricular systolic time intervals are presented in Table 4 The left ventricular STI ratio diminished between the first and third day of life This change was significant in the first few hours of life only For the rest of the observation period we found constant mean values (Fig 5)

Right ventricular STI changed markedly within the first days of life, especially the ratio RVPEP/RVET, which fell from values as high as 0 50 to an average of 0 28 within 6 days

Table 3 *Cardiac dimensions as a function of age* ($y=\ln(x)B+A$)

y=ordinate (dimension in mm) x=abscissa (natural logarithms of the age are expressed as e^x) SEE=standard error of the estimate, r=correlation coefficient p=probability

		SEE	r	p
End diastolic dimension of LV	$y=1\ 48e^x+15\ 76$	1 26	0 79	<0 001
End systolic dimension of LV	$y=0\ 85e^x+10\ 80$	1 05	0 71	<0 001
Left ventricular posterior wall	$y=0\ 28e^x+\ 2\ 97$	0 25	0 78	<0 001
Interventricular septum	$y=0\ 29e^x+\ 3\ 01$	0 28	0 76	<0 001
Aortic root diameter (Ao)	$y=0\ 70e^x+\ 9\ 30$	0 71	0 76	<0 001
Aortic valve separation	$y=0\ 57e^x+\ 6\ 90$	0 48	0 79	<0 001
Left atrial diameter (LA)	$y=0\ 74e^x+11\ 03$	1 14	0 66	<0 01
Pulmonary artery root dimension	$y=0\ 52e^x+10\ 62$	0 93	0 62	<0 001
Pulmonary valve separation	$y=0\ 48e^x+\ 7\ 90$	0 73	0 67	<0 001

Table 4 *Right and left ventricular systolic time intervals of the infants (mean ± 1 S.D)*

No = number of observations HR=heart rate, RVET=right ventricular ejection time, LVET=left ventricular ejection time, RPEP=right ventricular preejection period, LPEP left ventricular preejection period

Age	No	HR	PREP	RVET	PREP/RVET	LPEP	LVET	LPEP/LVET
0-24 hours	21	136±18	74±10	194±30	0 39±0 08	64± 9	195±24	0 32±0 04
3 days	21	126±15	63± 8	208±19	0 30±0 04	60±10	196±21	0 30±0 05
6 days	21	132±14	58±10	205±18	0 28±0 05	60± 7	193±13	0 30±0 04
1 month	20	155±10	51± 7	193±12	0 26±0 04	55± 7	184±11	0 29±0 03
2 months	20	150± 9	55± 7	204±14	0 27±0 04	59± 7	190±10	0 30±0 04
6–12 months	15	140±34	56± 8	236±15	0 23±0 03	63± 8	212±19	0 29±0 03

Fig 6 shows data for all patients, the first values being taken within 24 hours of birth The decline of RVPEP/ET ratio follows a parabolic function The changes are especially impressive when the first measurement takes place within the first 2 hours of life Fig 7 shows serial studies in 6 such patients The decrease of RVPEP/ET occurred between the first and the third day of life, thereafter, the decrease was slow Changes were minimal after the age of two months

DISCUSSION

In several previous publications echocardiographic measurements of cardiac structures, obtained in cross-sectional studies, were correlated with body surface area or weight. They appeared to follow either a linear or an exponential function (10, 11, 15)

The present investigation is, to our knowledge, the first longitudinal study on a cohort of newborns followed through the first year of life Such a longitudinal study should allow for a better demonstration of the relative changes of cardiac chambers with increasing age and weight

In our statistical analysis, LV, LA, Ao diameters, as well as septal and left ventricular posterior wall thickness were found to follow a linear correlation with age and weight. The

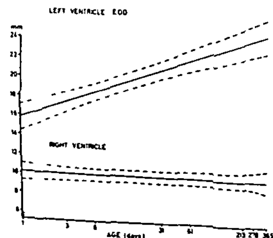


Fig 3 Echocardiographic dimensions of LV and RV at end-diastole in mm are plotted *against* the natural logarithm of age The regression lines (solid line) are plotted with their confidence limits

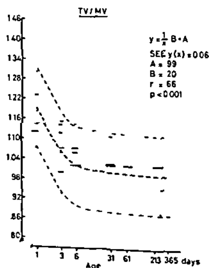


Fig 4 Sequential representation of the amplitude of the tricuspid valve (TV)/mitral valve (MV) *ratio* Each dot represents one measurement The dotted lines represent the regression line ±2 S E of the estimate for the total of observations

Table 2 *Cardiac dimensions as a function of weight (kg)*

SEE=standard error of the estimate r=correlation coefficient p=probability y=ordinate (dimension in mm) x=abscissa (weight in kg non logarithmic)

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End diastolic dimension of LV	y=1 38x+13 42	1 91	0 83	<0 001
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[illegible]	y=0 63x+ 5 91	1 28	0 70	<0 001
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[illegible]	y=0 55x+ 9 54	1 01	0 75	<0 001
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value ±1 S D was calculated Equations relating dimensions to weight are given in Table 2 The increase in dimensions of LV, LA, Ao, as well as LVPW, IVS and RVAW with body weight are best described as a linear regression

Because some measurements may vary as a function of age rather than weight, especially those related to the changes in circulation after birth, echocardiographic dimensions were correlated with age The equations of the corresponding regression lines (dimension versus log of age) are presented in Table 3 for each diameter These essentially show that LA, LV, Ao and the ventricular walls grow linearly with the natural log of age whereas the RV dimension does not change (Fig 3) Thus RV/LV decreases with age, this change is best demonstrated using the ratio tricuspid valve/mitral valve amplitude (Fig 4) and fits a parabolic function The LA/Ao ratios could also be characterized by a parabolic function during the first six days of life From the 6th day on they became constant For the total group a paired t test of LA/Ao ratios between the 1st and 3rd, and 3rd and 6th day was however, not significant

Left and right ventricular systolic time intervals are presented in Table 4 The left ventricular STI ratio diminished between the first and third day of life This change was significant in the first few hours of life only For the rest of the observation period we found constant mean values (Fig 5)

Right ventricular STI changed markedly within the first days of life, especially the ratio RVPEP/RVET which fell from values as high as 0 50 to an average of 0 28 within 6 days

Table 3 *Cardiac dimensions as a function of age* ($y=\ln(x)B+A$)

y=ordinate (dimension in mm) x=abscissa (natural logarithms of the age are expressed as e^x) SEE=standard error of the estimate r=correlation coefficient p=probability

		SEE	r	p
End diastolic dimension of LV	$y=1\ 48e^x+15\ 76$	1 26	0 79	<0 001
End systolic dimension of LV	$y=0\ 85e^x+10\ 80$	1 05	0 71	<0 001
Left ventricular posterior wall	$y=0\ 28e^x+\ 2\ 97$	0 25	0 78	<0 001
Interventricular septum	$y=0\ 29e^x+\ 3\ 01$	0 28	0 76	<0 001
Aortic root diameter (Ao)	$y=0\ 70e^x+\ 9\ 30$	0 71	0 76	<0 001
Aortic valve separation	$y=0\ 57e^x+\ 6\ 90$	0 48	0 79	<0 001
Left atrial diameter (LA)	$y=0\ 74e^x+11\ 03$	1 14	0 66	<0 01
Pulmonary artery root dimension	$y=0\ 52e^x+10\ 62$	0 93	0 62	<0 001
Pulmonary valve separation	$y=0\ 48e^x+\ 7\ 90$	0 73	0 67	<0 001

Table 4 *Right and left ventricular systolic time intervals of the infants (mean ± 1 S D)*
No = number of observations, HR = heart rate, RVET = right ventricular ejection time, LVET left ventricular ejection time RPEP = right ventricular preejection period, LPEP = left ventricular preejection period

Age	No	HR	PREP	RVET	PREP/RVET	LPEP	LVET	LPEP/LVET
0–24 hours	21	136±18	74±10	194±30	0 39±0 08	64± 9	195±24	0 32±0 04
3 days	21	126±15	63± 8	208±19	0 30±0 04	60±10	196±21	0 30±0 05
6 days	21	132±14	58±10	205±18	0 28±0 05	60± 7	193±13	0 30±0 04
1 month	20	155±10	51± 7	193±12	0 26±0 04	55± 7	184±11	0 29±0 03
2 months	20	150± 9	55± 7	204±14	0 27±0 04	59± 7	190±10	0 30±0 04
6–12 months	15	140±34	56± 8	236±15	0 23±0 03	63± 8	212±19	0 29±0 03

Fig 6 shows data for all patients, the first values being taken within 24 hours of birth The decline of RVPEP/ET ratio follows a parabolic function The changes are especially impressive when the first measurement takes place within the first 2 hours of life Fig 7 shows serial studies in 6 such patients The decrease of RVPEP/ET occurred between the first and the third day of life, thereafter, the decrease was slow Changes were minimal after the age of two months

DISCUSSION

In several previous publications echocardiographic measurements of cardiac structures, obtained in cross sectional studies, were correlated with body surface area or weight. They appeared to follow either a linear or an exponential function (10, 11, 15)

The present investigation is, to our knowledge, the first longitudinal study on a cohort of newborns followed through the first year of life Such a longitudinal study should allow for a better demonstration of the relative changes of cardiac chambers with increasing age and weight

In our statistical analysis, LV, LA, Ao diameters, as well as septal and left ventricular posterior wall thickness were found to follow a linear correlation with age and weight. The

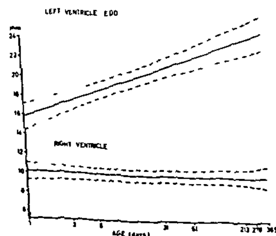


Fig 3 Echocardiographic dimensions of LV and RV at end-diastole in mm are plotted against the natural logarithm of age The regression lines (solid line) are plotted with their conf...

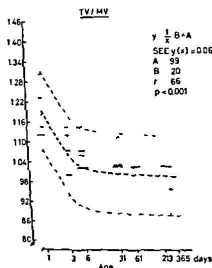


Fig 4 Sequential representation of the amplitude of the tricuspid valve (TV)/mitral valve (MV) ratio Each dot represents one measurement The dotted lines represent the regression line ±2 S E of the estimate for the total of observations

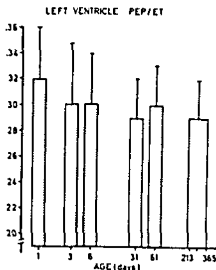


Fig 5 The mean values ±1 S D for the left ventricular systolic time interval ratio (LVPEP/LVET) are plotted as pillars for each age group

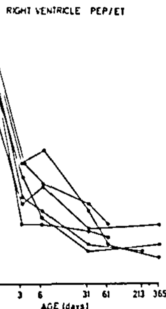


Fig 7 The sequence of RVPEP/RVET is drawn for 6 babies in whom the first echocardiogram was obtained during the first two hours of life

correlation coefficients are all significant (Tables 2 and 3) but smaller than in some previously published data (15, 19, 21, 27) thus indicating a larger variation of measurements This might be explained by several factors first, no sedation was used in these babies and, although care was taken to calm them, there were frequent position changes and spontaneous and respiratory movements Furthermore, the echocardiograph used did not have an automatic switched gain control Therefore the outline of the structures was not always absolutely clear (9) Nevertheless our mean values, as shown in Table 1, are within the range of previously published data obtained in cross-sectional studies, especially when inter laboratory differences are accepted (11, 15) International standardisation for the intra cardiac measurements, as has been recently suggested (30), may help in decreasing inter laboratory differences

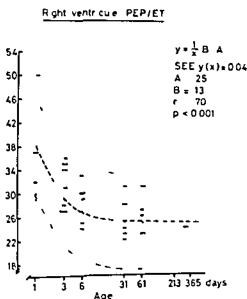


Fig 6 Sequential representation of the right ventricular systolic time interval ratios for all infants (RVPEP/ET) Each dot represents one measurement The first series of measurements (1 day) was in fact taken between 2 and 24 hours of life The dotted lines represent the regression line ±2 S E of the estimate for the total of observations

We decided to seek correlations with age since many changes occur as a result of post natal circulatory adaptation These adjustments to the changing hemodynamic situation take place within minutes, hours and weeks (1, 28, 29) and are clearly shown in this longitudinal echocardiographic study

Left ventricular growth is very marked while the right ventricle changes very little in the first months of life, indicating a differential growth pattern in response to the different hemodynamic load of the two ventricles after birth It has, however, to be kept in mind that the ultrasound beam passes only through a small part of the right ventricle RV dimension measured by M mode echocardiography

therefore does not provide an overall estimation of the real ventricular size, this echocardiographic measurement may also be influenced by configurational changes of the RV and by rotation of the heart within the chest

During the first hours and/or days of life, pulmonary expansion dilatation of the peripheral pulmonary arteries a decrease of pulmonary vascular resistance and a reversed ductal flow result in an increase in pulmonary blood flow and therefore an increase in pulmonary venous return with dilatation of the left atrium and the left ventricle immediately after birth During this time the babies' weight may decrease slightly or remain stable, but does not increase The increase in LA and LV diameter which we found on the echo is therefore independent of weight and due entirely to hemodynamic factors, at the same time the RV diameter does not change Later, the increase in LV diameter is related to increased body weight while the RV, slowly adapting to the low resistance in the pulmonary circulation does not follow the general growth pattern and lags behind

The relationship of the two ventricles can be expressed either by the RV/LV ratio or, as in this study by the TV/MV ratio (Fig 4) Statistical analysis revealed an exponential function for the first month and a constant value for the remainder of the first year of life The normal values for this relationship gained in the present study will be useful in the diagnosis of RV or LV volume overload and persistence of fetal circulation It must be remembered that the amplitude of the opening of the AV valves measured by echocardiography, is dependent on the ultrasonic beam really hitting the leaflet at the point where it has its greatest motion and is doing this perpendicularly to the leaflet This is certainly the case in RV volume overload, but also in the neonatal period The decrease in the TV/MV ratio could be partially due to configurational changes in the RV and to positional changes of the heart with age This, however, does not limit the value of this ratio, which should be a sensitive and early indicator of RV volume overload

The LA/Ao ratio has been shown to be useful for evaluating left to right ductus shunting in premature babies (7, 31), and it is equally helpful in determining left to right shunts in term infants (20) We therefore examined this relationship for our babies and found a ratio above normal in the first day of life decreasing rapidly to a normal value at day 3 It is likely that this is due to a transient left to right shunt through the ductus before closure When the ductus remains patent this ratio remains constant or may increase Normal values for this relationship will therefore be of help in diagnosing left to right shunts at ventricular or arterial level

Systolic time intervals can be determined accurately by echocardiography (13, 16, 17, 26) They are influenced by pre and afterload changes, by myocardial function and by various drugs (14, 35) In a normally functioning right ventricle without significant changes in pre load, RVSTI ratios should essentially reflect after load changes (12, 25, 26) We found a curvilinear relationship with time which is very similar to the decline of pulmonary artery pressures as demonstrated by hemodynamic studies on animals and in a few newborn babies (3, 38) Knowledge of the normal time course of the decline of pulmonary resistance gained by this method may be useful in determining a delayed fall in PA resistance, thus indicating persistence of fetal circulation (25), respiratory distress syndrome (12), or primary pulmonary hypertension, it may be possible to diagnose persistently raised pulmonary vascular resistance with a large VSD before clinical signs appear

Clamping of the umbilical cord with exclusion of the placental circulation results in an increase of systemic resistance immediately after birth (1, 29) The changes in after load, together with the increase in pulmonary circulation and LV pre load, are probably responsible for changes in the LV systolic time intervals However, LV contractility changes

after birth, as demonstrated by Friedman et al (16, 18), may also play a role These factors may explain the high values of LVPEP/LVET ratios found immediately after birth, subsequently, these values decrease rapidly and become stable The values found during the first year of life are lower than those in later life

Some of our babies were delivered by caesarian section It is possible that epidural or general anesthesia may influence cardiac function in these newborns Further studies are necessary to determine the effects of perinatal anesthesia

In conclusion, during the first year of life, growth of cardiac structures is related linearly to age with the exception of the right ventricle which does not change significantly in size during the first months of life Echocardiographically obtained LV and RVSTI accurately reflect changes related to postnatal adaptationtion over the first hours and days

Therefore, this method of non invasive investigation has a definite place in a neonatal unit for early detection of pathological changes

ACKNOWLEDGMENTS

The assistance of Mrs A Samai in typing the manuscript and of Mr D Furrer in preparing the figures is gratefully acknowledged This work was supported in part by the Swiss National Science Foundation no 3563 0 75 and the Emil Barrel Fund

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Submitted July 17, 1979
Accepted Sept 25, 1979

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Acta Pædiatr Scand 69 249–252, 1980

IRON RELEASE FROM THE STORES· A MECHANISM IN MAINTENANCE OF CONCENTRATION OF HEMOGLOBIN IN LOW-BIRTH-WEIGHT INFANTS

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sinki, [illegible]
of hemoglobin in low birth-weight infants. Acta [illegible] ... [illegible] at two months of age infants are dependant on
[illegible]
production This amount represents 5 mg per kg of body weight [illegible]
was mobilized from storage sites in an adult male under experimental conditions. Rapid
weight gain was associated with early depletion of iron stores. However, residual iron stores
[illegible]
with iron.

KEY WORDS Infants, low birth weight, iron

The body's ability to accumulate iron provides an internal reserve which can be mobilized when iron requirements exceed dietary supply Part of this iron is readily available from reticuloendothelial cells, the major part is more slowly mobilized from parenchymal cells (4) Under physiological conditions, erythropoiesis is considered iron-sufficient as long as storage iron is present However, patients with certain diseases, such as inflammations (3) and malignancies (9) may have sideropenia in the presence of iron stores. It has been postulated that this is due to impaired mobilization of iron from storage sites On the other hand, the reprocessing and release of iron is increased in other diseases, such as in the hemolytic anemias (1)

Rapid growth and co-occurringly stimulated erythropoiesis are unique features in infants after two months of age and are further accentuated in low-birth-weight infants Their erythropoiesis is dependent on storage iron due to the low iron content of their diet. In this study the efficiency of storage iron release was investigated in low-birth-weight infants.

SUBJECTS AND METHODS

A group of 57 low birth weight infants without major medical problems and with an average birth-weight of 1650 g and a total range from 1050 to 2000 g, were followed from two weeks to six months of age (6) Monthly blood samples were taken for laboratory studies including hemoglobin and serum ferritin The infants' diet consisted exclusively of milk during the first three months after birth Three different milk regimens were used breast milk, home-prepared cow milk formula or proprietary infant milk formula (Bona, ChymosOy, Lappeenranta, Finland), which was unsupplemented with iron After the age of three months fruit and vegetables were gradually introduced to the diet, consisting of one meal per day after four months and two meals per day after five months of age The infants were not given supplementary iron until there was evidence of iron depletion by arbitrarily

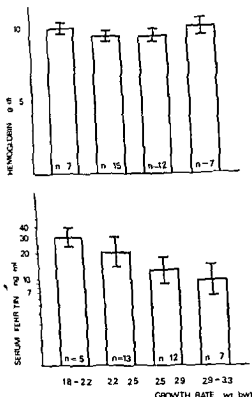


Fig 2 Mean values ± S E of hemoglobin and serum ferritin concentrations of low birth weight infants who were unsupplemented with iron at three months of age The infants are divided into four groups according to their rate of growth each group representing one standard deviation from −2 S D to +2 S D

tively high levels of serum ferritin, in individual infants or in the two groups with the slowest growth at three months of age, did not guarantee the level of hemoglobin or MCV obtained by iron supplementation (6)

DISCUSSION

The first weeks after birth are associated with a low rate of erythropoiesis and consequently with accumulation of storage iron primarily from senescent red blood cells The stored iron is then utilized by resuming erythropoiesis In this series there was evidence of iron deficient erythropoiesis in the presence of iron stores in low birth weight infants who received no supplementary iron These coexisting findings indicate that the rate of iron release from the storage sites could not maintain optimal erythropoiesis This might be partially due to impaired release of storage iron accumulated during fetal development This iron is estimated to be about 17 mg per kg at birth (8) Alternatively, high transferrin saturation during the neonatal period (6) may contribute to deposition of a substantial portion of the iron from senescent red blood cells in parenchymal cells, which is then reluctantly released Both of the above mentioned hypotheses would explain our data why iron deficient erythropoiesis may develop in the presence of iron stores in low birth weight infants

The precise mechanism of iron release from the storage sites is not known However, it is evident that it is regulated according to the needs of the bone marrow (4) Surprisingly, slight anemia in infants with still existing iron stores had not been able to facilitate the release of remaining storage iron although rapid growth was associated with early depletion of iron stores

In order to estimate the net flow of iron from the storage sites into the hemoglobin iron pool during the first months of life, we followed the hemoglobin iron in a group of infants These infants were almost exclusively on a milk diet with a constant iron intake and unsupplemented with iron beyond the time when iron stores were depleted In this series there were only five infants who fullfilled the above criteria and were followed at each age From two to four months of age the gain of 34 mg per month in hemoglobin iron was derived partly from the milk and partly from the iron stores The portion derived from the milk diet was probably no more than the 14 mg per month, accumulated in the hemoglobin pool from four to five months of age, when iron stores were practically depleted This amount would indicate approximately 50% absorption of the iron in unsupplemented milk and is in accordance with earlier reports on the exceptionally good availability of dietary iron by premature infants (2) The increased gain

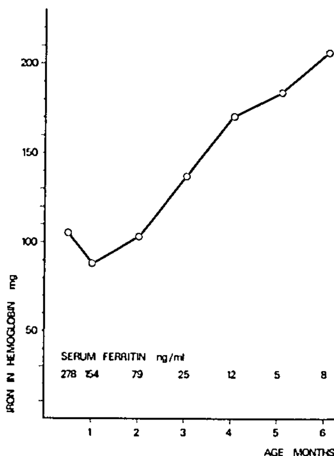


Fig 1 Accumulation of iron in hemoglobin during the first six months of life in five low birth weight infants who remained unsupplemented with iron during the whole follow up period Iron stores are indicated by mean values of serum ferritin concentrations

determined and age dependant levels of hemoglobin concentration The ones started on iron were subsequently excluded

Hemoglobin concentration was determined by Coulter Counter on capillary blood samples during the first 1–2 months after birth and on venous blood sample thereafter Serum ferritin was measured by radioimmunometric assay (7) The calculation of hemoglobin iron was based on the blood volume of 100 ml per kg at two weeks 85 ml per kg at one month and 73 ml per kg after one month of age (8) Individual rate of growth was evaluated by a ratio of actual body weight to birth weight

RESULTS

In growing infants increasing amounts of iron are accumulated in the hemoglobin pool It is derived from two sources from the iron stores and from the diet The sum of these two sources was followed by the estimation of monthly increments in hemoglobin iron in a group of five low birth weight infants, who were able to maintain exceptionally high levels of hemoglobin up to six months of age and could therefore by sustained without iron supplementation The gain was constant presented by a mean of 34 mg per month during the two month period from two to four months of age (Fig 1) In these infants the stores were depleted at four months of age as determined by a low concentration of serum ferritin After four months of age the gain in hemoglobin iron decreased to an average of 14 mg for the fifth month representing the iron that was derived only from the diet, although the continuing decrease in the mean ferritin concentration from 12 to 5 ng/ml during the fifth month may indicate some mobilization of storage iron During the period from five to six months of age increased amounts of iron were accumulated in hemoglobin

Since the growth rate and erythropoiesis were maximal from two to four months of age the demand for iron and the correlation between the individual growth rate and iron status were investigated in the whole group of unsupplemented low birth weight infants at the ages of three and four months The age of three months is more likely to be representative since the iron stores were practically depleted at four months Among the individual infants a great variation in weight gain was seen It ranged from 1 8 to 3 3 times the birth weight at three months of age (Fig 2)

There was a correlation between the concentration of serum ferritin and the rate of growth (Fig 2) A small weight gain was associated with higher serum ferritin values both at three months ($p<0\ 06$) and at four months ($p<0\ 04$) of age although serum ferritin concentrations had been similar in each of the weight gain groups at two weeks of age

No such correlation was found regarding the concentration of hemoglobin and the rate of growth either at three or at four months of age (Fig 2) Similarly there was no correlation between the mean corpuscular volume of red cells (MCV) and the growth rate

Residual iron stores, as indicated by rela

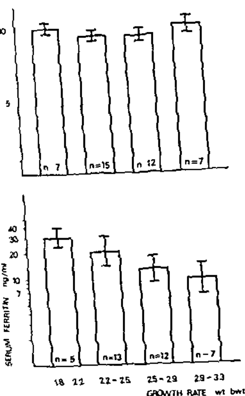


Fig 2 Mean values ± S E of hemoglobin and serum ferritin concentrations of low birth weight infants who

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from five to six months of age is probably due to increased quantities of solid foods in the diet According to these calculations, and bearing in mind that prior to the age of four months smaller quantities of milk were consumed, at least 20 mg of storage iron was mobilized during monthly periods at the age from two to four months This amount represents 5 mg per kg of body weight On the basis of these estimations, it seems that the flow of iron from storage sites in low-birth-weight infants is efficient and exceeds that found in adults By an experimental venesection it took one month before a deficit of 200 mg of hemoglobin iron was replaced by storage iron in an adult male (5) The 200 mg equals approximately 3 mg per kg of body weight

It appears therefore that the cause of anemia seen in the presence of residual iron stores in low-birth-weight infants is rather due to the exceptionally great need of iron for erythropoiesis than due to impaired mobilization of storage iron These findings emphasize the role of supplementary iron in preventing iron deficient erythropoiesis in low-birth-weight infants during the first months of life

ACKNOWLEDGEMENTS

This study was supported by grants from Foundation for Pediatric Research Finland, and Foundation for Chemical Research Finland

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Submitted July 23 1979
Accepted Sept 24 1979

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Acta Pædiatr Scand 69 253–254, 1980

LETTER TO THE EDITOR

VINCRISTINE IN THE TREATMENT OF POSTINFECTIOUS AND NEONATAL THROMBOCYTOPENIA

Sir,

Vincristine (VCR) has been used with varying degrees of success in *chronic* idiopathic *thrombocytopenic purpura* (ITP, when treatment with corticosteroids and/or splenectomy has failed (1, 2, 3, 4)

During the last two years we have tried vincristine in the most severe cases of *acute postinfectious* and of *neonatal* thrombocytopenia due to platelet antibodies The results have been encouraging in the patients treated so far (Table 1) All patients first received prednisone for at least 2 weeks prior to VCR, and a moderate dose of prednisone was continued during the VCR course VCR was given in doses of 1–1 5 mg/m² In the 2 patients with neonatal thrombocytopenia 1 injection was given, in the others 4 or 5 doses with intervals of one week

All 8 patients treated showed a significant rise in their platelet count in the first (6 cases) or second (2 cases) week after start of VCR No significant side effects of these relatively moderate VCR doses were observed In 4 of

Table 1 *Patients treated with vincristine (VCR)*

Diagnosis	Age	Duration before VCR	Platelet count before VCR	No of VCR injections	When increase in platelet *count was* observed (after start of VCR)	Total duration of *thrombo*-cytopenia before normalization	Remarks
1 Postinfect thrombo-cytopenia	1½ yrs	1½ mos	5 000–10 000	5	1st week	10 mos	
2 Postinfect thrombo-cytopenia	5 mos	3 mos	11 000	4	1st week	5 mos	
3 Postinfect thrombo-cytopenia	8 yrs	2 mos	5 000–10 000	5	2nd week	5 mos	
4 *Postinfect* thrombo-cytopenia	11½ yrs	2 wks	1 000– 4 000	5	2nd week	2 mos	
5 Postinfect thrombo-cytopenia	6½ yrs	4 mos	8 000	5	1st week	?	
6 Neonatal thrombo-cytopenia	Newborn	18 days	3 000–10 000	1	5th day	4 wks	
7 Neonatal thrombo-cytopenia	Newborn	14 days	8 000	1	4th day	4 wks	
8 Chronic ITP	10 yrs	>16 mos	10 000	5	1st week		Relapse *Remission* following splenectomy

the 5 patients with postinfectious thrombocytopenia the marked improvement observed was sustained after discontinuation of VCR and prednisone, and gradually a complete normalization of the platelet counts took place The fifth patient is also in full remission, but the observation period is still too short There was a rapid normalization of platelet counts in the 2 patients with neonatal thrombocytopenia The last patient (no 8), the only one with chronic ITP, relapsed 5 weeks after the course of VCR and was subsequently splenectomized with good result.

When evaluating these results one should of course bear in mind the tendency to spontaneous remission in postinfectious and neonatal thrombocytopenia However, the improvement in all our patients came in so close connection with start of the combined VCR–prednisone therapy that a causal relationship seems highly probable

In conclusion, VCR combined with moderate doses of prednisone can be tried in the most severe cases of postinfectious and neonatal thrombocytopenia due to platelet antibodies, when prednisone alone fails, i e in cases with severe bleeding tendency and platelet counts repeatedly in the range of 10000 or lower This form of therapy can also be tried in chronic ITP when both prednisone and splenectomy fail, or even before splenectomy is considered if for one reason or other (e g the patient's age) one hesitates to perform splenectomy

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Acta Pædiatr Scand 69 255–256, 1980

SHORT COMMUNICATION

OCCULT NEUROBLASTOMA PRESENTING WITH CEREBELLAR SYMPTOMS, RESULTS OF COMPUTED TOMOGRAPHY

The clinical relationship between occult neuroblastoma and myoclonic encephalopathy (syndrome of 'dancing eyes') (6, 1) or cerebellar ataxia (5) has been repeatedly recognised

Neuroradiological examinations have in general been unrewarding (1, 3) As no results of computed tomography in this condition have been published, we describe a child who had typical history and symptoms of cerebellar tumour, but no abnormality in the posterior fossa in computed tomography The symptoms subsided after an occult neuroblastoma had been removed from the thoracic cavity

Case report A two-year-old girl had previously been in good health In May 1978 her parents noticed some mild clumsiness but no medical examination was made However the symptoms increased steadily and in August the girl was very shaky things fell from her hands and she had been irritable in the mornings She had marked symmetrical ataxia some intention tremor and perhaps some degree of nystagmus Papilloedema was not seen Nevertheless a cerebellar tumour was strongly suspected The initial examinations were normal or inconclusive and when a computed tomography was performed no abnormality was seen in the posterior fossa or in the ventricular cavities There was a small area of decreased density on the right side under the basal ganglia but the possibility of a movement artifact could not be excluded In chest X ray a dense shadow was seen behind the heart This proved to be a ganglioneuroblastoma extending from Th V to Th X There was no evidence of metastases and the cathecholamines were normal The girl recovered uneventfully after operation Radiation therapy was initiated on the second postoperative day In the next evening the girl was observed to have clear fine spontaneous nystagmus but no "dancing" eye movements The nystagmus disappeared in two days The computed tomography was

In published cases the cerebellar symptoms secondary to an occult neuroblastoma have mostly been of the type of myoclonic encephalopathy (1), but ataxia is also known as the mode of presentation (5, 7) No good explanation has been offered for these paraneoplastic phenomena (8) and in particular the catecholamines have often been normal (1) Also the computed tomography failed to explain the cerebellar symptoms in our case, the questionable lesion under the basal ganglia on the right side had rather given rise to opsoclonus ("dancing eyes") (2) which was *not observed in our patient* The history and symptoms of our patient resembled closely those of a cerebellar tumour, the subacute course was entirely different from "acute cerebellar ataxia" also known to occur with occult neuroblastoma (5) Clinically it is important that in most cases the "occult" neuroblastoma is relatively benign (1) and situated within thoracic or abdominal cavity (5, 3) Therefore, an active search is indicated in cases where cerebellar symptoms remain otherwise unexplained

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Acta Pædiatr Scand 69 257–258, 1980

SHORT COMMUNICATION

OBSERVATIONS ON THE PROSTATIC UTRICLE IN THE FETUS AND INFANT

The prostatic utricle (uterus masculinus) is a structure which is not frequently mentioned in the literature It is situated within the colliculus seminalis (verumontanum) and represents the remaining ends of the united Muellerian ducts It is usually present as a small sac a few millimetres long, which opens in the centre of the colliculus seminalis through a small slitlike opening

In the fetus the utricle and the glands in its vicinity are the first structures to be affected by continuous and intense oestrogenic stimulation and early metaplastic changes are taking place in its epithelium Gradually, in later months of gestation, the metaplastic cells become squamous and are eventually shed, thus leading to lumen formation Desquamated squamous cells and cellular debris are then discharged into the prostatic urethra through an opening which is usually small and slitlike (Fig 1) Normally, a gradual regression of metaplastic changes takes place after birth with the cessation of oestrogenic stimulation and this leads to a gradual decrease in the distension of the utricle This is however, not always the case and certain complications may arise (13) A bulging colliculus seminalis, containing a very large utricle or a utricular cyst may interfere with outflow from the bladder Such urinary retention by a distended utricle or utricular cyst may persist in a newborn for several days Catheterisation of the cyst may cause the infant to pass urine immediately Secondary changes in the upper urinary tract like hydroureter, hydronephrosis and pyonephrosis may also occur, if the condition is not treated (1, 3, 5, 9) In older children, the edges of the utricle can be firmly fixed together or even joined by fibrous tissue, or the colliculus seminalis may be strongly bulging, thus causing stenosis of the prostatic urethra These conditions may require surgical procedures Utricular enlargement and cysts have been reported to occur in children and in adults (6, 4) They can be demonstrated by utriculography, by cystogram and various other diagnostic procedures (2, 4, 7, 9, 10)

Cystic dilatation of the prostatic utricle is rare in male children with otherwise normal genitalia It can form a reservoir of infection and a site of stone formation in the posterior urethra The condition can be treated by conservative and surgical methods (8, 10) A tubular diverticulum is often seen in connection with the Prune Belly Syndrome It does not seem to cause particular symptoms and does not require treatment (12)

In a large survey on prostates from fetuses, neonates and infants (13), we noted that the utricle was sometimes markedly enlarged, in one case to an extent of 11 mm instead of the average of 1–5 mm Polse & Edelbrock (9) mention as possible causes obstruction of the utricle by valves, fused fetal membranes or desquamated epithelium at the point where the utricle enters the urogenital sinus Urinary retention may well have occurred in some of our cases where a large utricle had caused extreme bulging of the colliculus seminalis into the urethra, but in view of their short time of survival this would not have been noted clinically

According to Howard (5), utricular enlargement may occur as a sign of defective virilisation of the male embryo He pointed to the association of utricular enlargement and various types of hypospadias Wells et al (11) came to similar conclusions in their experi-

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CASE REPORT

HYPERTHYROIDISM, DIABETES MELLITUS AND THE CONGENITAL RUBELLA SYNDROME

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ABSTRACT Floret, [illegible] Infantile B Hopital [illegible] dental enamel and [illegible] was treated success[illegible] premature craniosynostosis and a craniectomy was performed at the age of [illegible] years. [illegible] diabetes mellitus developed at 17 years and was well controlled by insulin therapy. Histocompatibility (HLA) antigens were A2, B8, B40 Diabetes mellitus and thyroid disorders have previously been reported after congenital rubella, and recently after congenital cytomegalovirus infection Our patient had both endocrinopathies. It is possible that HLA B8 antigens might be responsible for increased susceptibility to rubella infection.

KEY WORDS Congenital rubella syndrome, Graves' disease, diabetes mellitus

Endocrine disorders have been recently documented among the various complications of congenital rubella Diabetes mellitus has been the most commonly noted Thyroid dysfunction particularly hyperthyroidism which is extremely uncommon in young children, has also been reported

CASE REPORT

Gerard was born at term in December 1960 Delivery was uneventful Birth weight was 3050 g There was no family history of diabetes mellitus and thyroid disorders He was the second of 2 children and his mother developed a rash after fairly close contact with her older son who had rubella [illegible] was [illegible] of [illegible] red [illegible] age [illegible] tea

Hyperthyroidism with nervousness fever, tachycardia (pulse rate 160/min) bilateral exophtalmos with lid retraction and a diffuse, vascular goiter His height was normal (100 cm) but his weight was below the third percentile for age (13 9 kg) The bone age was 7 1/12 years. Laboratory tests gave the following results serum cholesterol, 4 27 mmol/l Hormonal iodine 756 48 nmol/l (normal 266 34±36 25 nmol/l) ^{131}I uptake was elevated (75% at 2 hours, 84% at 4 hours, 76 5% at 24 hours) and there was no suppression after triiodothyronine administration (75 µg/day for 8 days)

Premature craniosynostosis with microcephaly (head circumference 46 5 cm) The skull roentgenogram showed oxycephaly with complete synostosis of the longitudinal and transverse sutures Fundus examination initially showed no papilledema However bilateral [illegible]lledema [illegible] of the [illegible] (I Q = [illegible]), delayed speech bilateral sensory neural deafness, hypoplasia of the [illegible] the teeth, typical [illegible] rubella hemag[illegible] was 1/32 [illegible] therapy was instituted and led to euthyroidism Treatment was withdrawn gradually and stopped after 4 1/2 years without reappearance of thyrotoxic symptoms Laboratory tests in June 75, at the age of 15 years, were normal PBI 496 44 nmol/l (N = 370 36±102 44 nmol/l), triosorb 29 4% (N = 30±3%), tetrasorb 83 85 nmol/l (N = 51 6 to 141 9 nmol/l)



Fig. 1 Neonate aged 32 weeks Still born Prostate The utricle is filled with metaplastic cells The posterior wall of the urethra shows marked squamous metaplasia The section shows the opening of the utricle into the urethra *Ut* Utricle *U* urethra HE ×60

ments on castrated rat fetuses Growth of the necessary genital structures was retarded and sometimes caused development of an abnormally large utricle and sometimes hypospadias The effects could be prevented by implanting testosterone in the castrated fetus The presence of a very large and extended utricle in some of our cases with hypospadias may, therefore, have been due to some lack of androgenic hormone (14)

In conclusion it may be stated that urinary obstruction of unknown origin in the newborn may be due to enlargement of the prostatic utricle, either alone or in conjunction with the colliculus seminalis Associated changes in the upper urinary tract may also occasionally be found Early recognition and treatment of the condition is, therefore, indicated

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exclusively Furthermore, a majority of authors believe that a viral agent alone is not responsive for diabetes mellitus, and that genetic predisposition is essential for increasing the susceptibility of children to viral infection In support of this view, 70% of children with congenital rubella and diabetes mellitus have HLA B8 antigen (6) HLA B8 antigen has also been found in 35% of patients with hyperthyroidism, against 16 3% in controls (7)

Besides our case, only one reported patient with a congenital viral infection and a thyroid disorder (11) has had HLA typing it is noteworthy that in both cases HLA B8 antigen was found This provides an additional argument for a viral etiology in some thyroid disorders in children

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Submitted May 25, 1979
Accepted Oct 1, 1979

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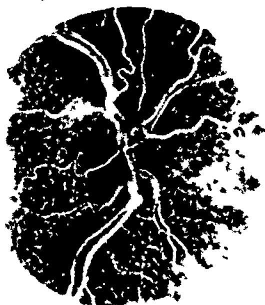


Fig 1 The patient s fundi showing congenital rubella retinopathy

The peak TSH was 8 5 *μU/ml* after I V injection of 200 μg TRH

In March 78 at the age of 17 years the patient develloped diabetes mellitus with massive glycosuria (688 2 mmol/24 h) good control was obtained by the use of *regular and protamine* zinc insulins The HLA phenotypes was found to be A2 B8 B40 There were no detectable serum antithyroid antibodies (antithyroglobulin anti mi*crosome* anti colloid)

DISCUSSION

In the present case, serological evidence of *congenital rubella* was not available However, in view of the history of maternal rubella and the defects noted (deafness typical retino pathy, mental retardation, dysplasia of the teeth) this diagnosis appears highly probable Premature craniosynostosis was also present in our patient This is unusual in congenital rubella and must probably be regarded as a complication of hyperthyroidism (4 8)

The existence of a relationship between diabetes mellitus and congenital rubella was first established by Forrest et al (5) Cooper (2) also noted that the incidence of diabetes mellitus was at least four times greater in children with congenital rubella, compared with the general population Menser et al (9) reported the occurrence of diabetes mellitus in 20% of 45 patients with congenital rubella One of these patients, who developed diabetes mellitus at 28 years, also had a period of hyper thyroidism at the age of 16

Thyroid disorders following congenital *rubella* are less well documented Zinng et al (12) reported a case of congenital rubella with thyrotoxicosis which appeared at the unusual age of 3 years In addition hypothyroidism with chronic lymphocytic thyroiditis has been described in two children aged 4 years and 5 *3/12 years respectively (1, 13)*

To our knowledge, only Menser s patient (9) and our own have both endocrine defects This association between congenital rubella diabetes and thyroid disorders *may not be* for tuitous *However the pathogenesis of* the endocrine defects remains unclear Pancreatic localization of rubella virus has been demon strated experimentally (9) and identified in the human fetus (2 3) Similarly Zinng et al (13) have detected *rubella virus* antigen in the *germinal centers of lymphoid follicles* obtained by thyroid biopsy in a patient with thyroiditis and congenital rubella

Thyrotoxicosis (10)—and more recently diabetes mellitus (11)—have been reported after congenital cytomegalovirus infection therefore the causative agent in these endo crine disorders may not be the rubella virus

Acta Pædiatr Scand 69 263–267, 1980

CASE REPORT

AN ARTHROPATHIC FORM OF OSTEOGENESIS IMPERFECTA

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ABSTRACT Penttinen, R , Sipola, E , Kouvalainen, K , Simila, S and Remes, M (Department of [illegible] University of Turku, and Department of Paediatrics, University o [illegible] 1980.— [illegible] joint dis[illegible] prolinemia and hydroxyprolinuria Rheumatoid arthritis was highly unlikely Anamnestic data revealed two long bone fractures Collagen biosynthesis was studied in fibroblasts cultured from the patient s skin Chromatograms of ^{3}H labelled culture media proteins on ion exchange celluloses revealed an increased ratio of type III collagen to type I collagen when compared with the chromatograms of age-matched control fibroblasts This finding is typical of certain cell strains in osteogenesis imperfecta The patient might thus express a new variety of osteogenesis imperfecta with chronic arthropathy

KEY WORDS Collagen biosynthesis, juvenile rheumatoid arthritis, osteogenesis imperfecta, osteoporosis

Osteogenesis imperfecta (OI) is a heritable disorder of connective tissue which principally affects bone (8 9) Other signs of OI include opalescent teeth, thin skin, short stature, blue sclerae and dislocated tendons, although not all patients express all of these symptoms (8) Classically, the OI syndrome has been divided into OI congenita and OI tarda, the former being characterized by a lethal course in its most severe form Another variety of congenital OI leads to severe skeletal impairment, but is not lethal OI tarda is considered benign (8 9) The colour of the sclera is variable Patients having white sclerae express severe bone disease whereas those with blue sclerae often have milder skeletal findings (3) OI can also be classified into severe, moderate and mild based on the bone findings (2) Recently, OI was divided into four types on the basis of the pattern of inheritance, clinical signs and severity of the symptoms (11)

Ion-exchange cellulose chromatograms of the collagens synthesized by skin fibroblasts separate the OI patients into three major varieties (10, 12) Two of them are characterized by an impairment of type I collagen synthesis, which leads to an increased ratio of type III to type I collagen synthesis in the cultures In the first biochemical variety (OI congenita, lethal type), type III collagen comprises 40% to 50% of the total collagens In the second variety (OI congenita and tarda cases), type III collagen makes up 25% to 30% of the total collagens, whilst in the third biochemical variety (OI congenita and tarda cases) no alterations in the level of type III from total collagen synthesis (normally 10% to 15%) are observed (12)

Recently, a patient suffering from pain, swelling and stiffness in several joints was admitted to Oulu University Central Hospital for suspected rheumatoid arthritis Subsequent clinical studies did not confirm this diagnosis The patient had generalized osteoporosis, and the medical history revealed two fractures Skin fibroblasts were cultured for studies on

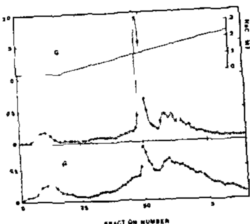


Fig 2 DEAE cellulose chromatogram of ³H proline labelled proteins of the patient's cell culture medium (lower panel) and the medium of an age matched control culture medium (upper panel) The procollagen type I peak at fractions 49–53 is much smaller in the patient's chromatogram than in the control chromatogram especially when compared with the procollagen type III peak regions (58–62) The start of the gradient (from 0 to 0 22 M NaCl) is indicated with arrows

or other general symptoms At the age of fourteen her height is 170 cm her skin appears normal but the teeth are opalescent and extensive caries has been a problem The sclerae are white and the patellar and achilles tendons are normal

Laboratory findings

The erythrocyte sedimentation rate has been normal since the first symptoms the maximal recording was 16 mm/h Red white blood cell and thrombocyte counts have been normal The serum immunoglobulin IgG IgM IgA IgE values and liver function tests have also been normal Serum calcium concentration has varied between 2 30–2 40 mmol/l phosphorus 1 3–1.5 mmol/l and alkaline phosphatase 52–366 U/l Urinary excretion of mucopolysaccharides and amino acids were within normal limits with the exception of hydroxyproline which amounted to 471 1068 μmol/day (the upper normal limit is 370 μml/day)

The free hydroxyproline of serum was also elevated (12 2 21 4 μmol/l normally 11 8 μmol/l) Serum urate was 270 μmol/l (normal 340 μmol/l) Serological tests for juvenile rheumatoid arthritis (JRA) including the latex agglutination test and tests for antinuclear antibodies and other circulating antibodies have been negative throughout the disease process AST was below 36 and ASTA was 2 2

Radiological findings

Signs of osteoporosis were detected in ankle and wrist bones already at the age of 10 years The signs were striking at the age of 14 years in all parts of the skeleton the cortex being very thin Severe destruction of the wrist and ankle joints terminated to anchyloses (Fig 1) but the changes were less pronounced in other parts of the skeleton

COLLAGEN STUDIES

Methods of cell cultures

Forearm skin fibroblasts were cultured, using routine methods (10) For studies on collagen synthesis confluent cultures at the 5th to 7th passages were labelled for 24 hours with 20 μCi/ml of ³H G proline together with 50 μg/ml of β aminopropionitrile and 50 μg/ml of ascorbic acid, in the absence of serum The medium and cell layers were separated, treated with protease inhibitors (10), and dialyzed for 72 hours against distilled water at +4°C Procollagen chains of one half of the media were separated with DEAE cellulose chromatography (10, 12) The other half of the media and cell layers were recombined and digested with pepsin at +4°C for 6 hours Type specific collagen chains were separated with CM cellulose chromatography in 4M urea (10, 12)

Results

The chromatograms show a decrease in the synthesis of procollagen type I (fractions 49–53, Fig 2, lower panel) compared with the control culture (upper panel) After the conversion of procollagens to collagen like molecules with pepsin and the separation of the collagen chains with CM cellulose, an increase in collagen type III was observed in the OI culture (Fig 3, upper panel) when compared with the pattern of an age matched control culture (lower panel) These results are similar to those published (10, 12) on the majority of the OI fibroblasts studied and refer to the second biochemical type of OI

DISCUSSION

We present here a patient having an arthropathy resembling JRA Severe osteoporosis, thinning of the cortex and severe destruction

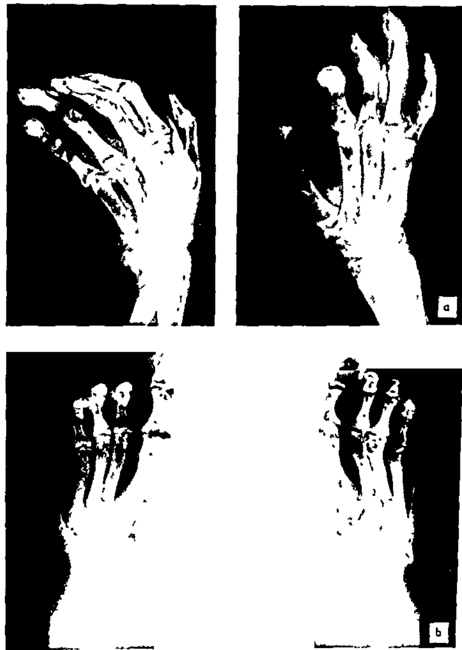


Fig 1 X ray pictures of (*a*) the wrist, and (*b*) the ankles of the patient at the age of 14 years Severe osteoporosis is noted Fusions have developed in the regions of wrists and ankles Epiphyseal resorption is almost complete

collagen metabolism The results of the chromatographic data were analogous to those seen in the second biochemical variant of OI

CASE HISTORY

The patient is the 3/5 child of healthy unrelated parents No symptoms similar to those seen in the patient have been observed in the siblings She was born after an uneventful pregnancy, the birthweight was 3 100 g The neonatal period and psychomotor development was normal

At the age of six years she had a traumatic fracture of her left forearm which healed in a normal way Four years later the left arm was distended During the healing the wrist became tender and swollen, and within a few weeks

After three

juvenile rheu

cotherapy consisting of acetylsalicylic acid (65 mg/kg/day) and chloroquine (5 mg/kg/day) were started but in spite of this she developed anchylosis of the left ankle During the first year of the disease, the right wrist, both shoulder joints and several metacarpo phalangeal and interphalangeal joints became involved A supracondylar fracture of the left humerus resulted from a minor fall when she was 12 years old During the next two years her joint symptoms progressed She became unable to clench her left wrist, and several of the minor joints of her hands became completely anchylotic The right hand was only slightly better Impaired function was present also in the elbow, shoulder and ankle joints However, she did not need assistance for any daily activity During the course of the disease she never had periods of fever

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Submitted July 3 1979
Accepted Sept 11 1979

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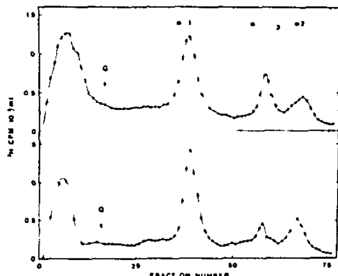


Fig 3 CM cellulose chromatograms of pepsin treated ^{3}H labelled proteins (medium plus cell layer) of the patient (upper panel) and the age matched control culture (lower panel) The start of the gradient (from 0 to 0 11 M NaCl) is indicated with arrows The proportion of type III collagen (from the sum of alfa 1 (I) plus alfa 2 chains representing type I collagen) is increased from ca 14% in the control chromatogram to ca 31% in the patient s chromatogram

in the bones of the wrists and ankles were present Laboratory tests showed no abnormalities of parathyroid function The skeletal fragility with spontaneous fractures pointed, however, to osteogenesis imperfecta

Studies on collagen synthesis indicated a relative increase in type III collagen synthesis similar to that observed in many OI skin fibroblast cultures Abe et al (1) noted that normal fibroblasts seeded in high density produce more type III collagen than those seeded in low density In the present experiments confluent cultures were used, and the results cannot be explained on the basis of small variations in cell densities of such cultures Vuorio (13) using a similar chromatographic approach did not find alterations in the ratios of collagen types in skin and synovial cell cultures from rheumatoid arthritis and control patients In our experiments on several inherited and acquired connective tissue diseases only cells from OI patients have thus far shown an elevation in type III collagen synthesis The severe bone findings of our patient and the results from the collagen analysis indicate to us that she might suffer from an atypical form of OI tarda

The frequency of OI is about 1 in 40000 births (4) whilst that of JRA is about 1 in 30000 children (6) Accordingly, the simultaneous occurrence of both these diseases should be very rare, i e ca 1 2 10^{8} children The diagnosis of JRA is improbable because of atypical clinical findings and negative laboratory tests Urinary excretion of hydroxyproline is variable in OI (see 5, 7 for references) It is elevated in certain ' collagen diseases , such as lupus erythematosus, periarteritis nodosa or rheumatoid arthritis (5) and might in our patient be derived from her extensive cartilage and bone destruction

The reason for the arthritic component in this case is obscure It can be speculated that the defect was not limited only to type I collagen, but was also present in type II collagen causing the destruction of cartilage

ACKNOWLEDGEMENTS

We thank Dr Anna Liisa Makela from the Department of Pediatrics
on the manu
tographic ar
corrected by Miss Jia Newcombe B Sc This work was supported by a research contract from the Medical Research Council of the Academy of Finland

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Acta Pædiatr Scand 69: 269–271, 1980

CASE REPORT

SEVERE POLYARTHRITIS FOLLOWING CAMPYLOBACTER ENTERITIS IN A 12-YEAR-OLD BOY

A. N. BÉKASSY,[1] H. ENELL[1] and C. SCHALÉN[2]

From the Departments of Paediatrics[1] and Medical Microbiology,[2] University Hospital, Lund, Sweden

ABSTRACT. Bekassy, N. A., Enell, H. and Schalen, C. (Departments of Paediatrics and Medical Microbiology, University Hospital, Lund, Sweden). Severe polyarthritis following Campylobacter enteritis in a 12 year-old boy. Acta Paediatr Scand, 69: 269, 1980.—A severe attack of acute polyarthritis following a verified Campylobacter jejuni enteritis is described in a 12-year-old boy. The patient possesses the HLA-B27 antigen—often found in postinfectious arthritis following acute enteric infections. The ASO titre showed a significant rise, but other serological findings and the clinical course made streptococcal infection unlikely. Investigations to elucidate cross-reactivity between the two micro-organisms turned out negative.

KEY WORDS: Polyarthritis, Campylobacter, HLA-B27, ASO-titre

[T]he HLA-B27 antigen has been found in high [f]requency among patients with Mb Bechterew, [M]b Reiter, psoriatic arthritis (13, 14) and in [p]ostgonococcal arthritis (1). It is also known [t]hat postinfectious arthritis, as a complication of acute enteric infections, almost exclusively arises in individuals carrying the HLA-B27 antigen, such data have been reported concerning Salmonella (7, 8, 10), Shigella (4) and *Yersinia enterocolitica* (1, 13). Recently, Berden et al. (2) described the occurrence of polyarthritis in an adult man with a preceding *Campylobacter jejuni* infection.

CASE REPORT

On September 3, 1978, a 12 year-old boy was admitted to the Department of Paediatrics with fever and pain in several joints of one day's duration. Previously, at the age of 6, he had suffered from acute unilateral coxitis of unknown etiology; otherwise he had been essentially healthy. He had had a four-day period of diarrhoea [illegible] ... All X-ray showed periarticular soft tissue swelling of affected joints and, one month later, signs of slight decalcification.

Despite oral salicylate 100 mg/kg/d, high fever persisted and tenderness of all finger and toe joints increased in the first week of observation. On September 14, prednisolone 20 mg/d was added and divided into two doses. This alleviated the articular symptoms, but fever at about 38°C remained for another month. A 10-day course of oral penicillin V was initiated on September 28, without any *clinical effect*. Attempted reduction of prednisolone caused clinical exacerbation. Chloroquine phosphate, 0.16 g/d, was added. The boy subsequently improved and was allowed to go home on October 20, with gradually tapering doses of prednisolone, which was discontinued after 4 months. Chloroquine phosphate and salicylate were discontinued after another fortnight. The boy is currently free of symptoms and has been without medication since January 27, 1979.

Laboratory data. The WBC ranged 20–30×10^9/l with about 90% polymorphonuclears. The platelet count ranged 400–630×10^9/l. The ESR was 125 mm/h; serum acute phase reactants were markedly elevated. Complement component levels were normal. Serum IgG was 20 g/l, IgM 1.45 g/l. A transient erythrocyturia of about 100 to 500×10^6/l cells and shedding of epithelial cells, some of which were in casts, lasted no more than about 10 days after admission. The ESR fell to normal within 4 months as did the other laboratory parameters including WBC, IgG and IgM

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CASE REPORT

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Table 1 *Titres against streptococcal and campylobacter antigens and occurrence of anti IgG i consecutive serum samples*

Date	ASO	ADNAse	ASH	Campylobacter agglut titre (own strain)	Precipitating anti IgG
Sept 4	185	37	<2 000	1/160	–
Sept 11	260	37	<2 000	1/1 280	–
Oct 4	875	<37	<2 000	1/320	–
Nov 2	610	<37	<2 000	1/160	+
Dec 6	480	<37	<2 000	1/80	+
Febr 2	275	<37	<2 000	1/20	–

values The patient had the following histocompatibility antigens HLA-A3, 9, B18, 27, Cw1

Bacteriological and virological findings On September 4, *Campylobacter jejuni* was isolated from the faeces Subsequent faecal specimens were negative for Campylobacter and other common pathogens ECHO 30 virus was also isolated from faeces on September 4 Blood and urine cultures yielded no bacterial growth From repetitive throat swabs no streptococci were cultured No positive stool cultures were obtained from family members, nor from their dog or parakeets

Serological findings Campylobacter serology was done with the patient's own formalinized strain The agglutination titre rose from 1/160 on September 4 to 1/1 280 on September 11 and later fell successively (Table 1) Yersinia type 3 and 9 serology was negative The was no rise in the antibody titre against ECH 30 In addition, the ASO titre determin according to Fasth (6), rose significantly fr 185 U/ml to 875 U/ml during the first mo of disease (Table 1) The ADNAse titre (B ringwerke AG, W-Germany) did not r neither did the anti strepto hyaluronid (ASH) titre Waaler Rose test and ANF w repeatedly negative Gel precipitating a immunoglobulin antibodies were dem strated in serum samples from Novemb and December 6, by the use of a rece described immunodiffusion method u aggregated human IgG (15)

In order to elucidate whether cross r tivity between Campylobacter and streptol 0 might occur, five paired serum samples f patients with Campylobacter enteritis examined No significant rise of ASO t was found Moreover, of 19 convalescent drawn from other patients one to three w after onset of Campylobacter enteritis two had titres above 200 U/ml 260 and U/ml respectively

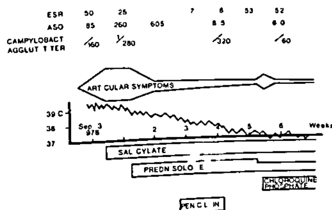


Fig
and
ASO –

DISCUSSION

With the history of diarrhoea preceding polyarthritis the isolation of *Campyloba jejuni* from faeces, and the observed antib titre change against the homologous st a postinfectious arthritis triggered by Campylobacter infection appears lik

However, the ASO titre change alone would provide substantial evidence for a streptococcal infection to have occurred at about the same time, although ADNAse and ASH titres were not elevated. Nevertheless we regard post streptococcal arthritis unlikely as there were no clinical signs of actual streptococcal infection and repeated throat cultures were negative. Furthermore the arthritis was not of the migrating type as in rheumatic fever. It is of some interest that no association with any particular histocompatibility antigen has been found so far in rheumatic fever (11).

The antibody titre changes recorded were thus compatible with an actual infection caused by either Campylobacter or streptococci. From the limited investigations on sera from other patients we obtained no evidence for any cross reactivity between Campylobacter and streptolysin O. It is known that anti immunoglobulin antibodies may interfere with various agglutination titre determinations (3, 12); recently we have described an influence of anti IgG in human sera on the precipitation in gel of streptococcal peptidoglycan (16). However ASO titre determination is not known to be influenced.

The transiently raised cell number in the urine may be due to a urinary tract infection as this has been documented recently for Campylobacter (5), but routine urine cultures remained sterile. There was no other clinical or laboratory evidence of acute glomerulonephritis. Absence of conjunctivitis and dysuria exclude Reiter's syndrome.

We are inclined to ascribe the acute polyarthritis in our patient to the preceding Campylobacter infection. We find it desirable that data including streptococcal antibody titres are given in case reports of this category of diseases. Information on HLA antigens will also be of interest for comparison with other patients who suffer from arthritis related to intestinal pathogens, since strong associations with the HLA antigen B 27 have been reported in Salmonella [illegible] infec

ACKNOWLEDGEMENTS

The authors express their gratitude to B. Löw, Blood Centre, University Hospital, Lund, for tissue antigen typing; to J. Henrichsen, Rigshospitalet, Copenhagen, for determination of ASH titre and to H. Svantesson, Department of Rheumatology, University Hospital, Lund, for very useful clinical considerations.

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Submitted Aug. 7, 1979
Accepted Oct. 4, 1979

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To Niilo Hallman at his reception of the Rosén von Rosenstein medal, May 1979

Niilo Hallman

It is a great pleasure and honour for me to welcome you here today in Uppsala as a most distinguished guest of the Swedish Pediatric Association and as a receiver of the Rosen von Rosenstein awards

It is easy to introduce Niilo Hallman to this audience because in the Scandinavian pediatric setting he is since many years a leading and wellknown person, both in his capacity as an outstanding research worker with a comprehensive scientific activity and as a leading member and adviser of many of our scientific societies, editorial boards and other organizations in the fields of pediatrics and child health

A rapid and active university career led Niilo Hallman to the chair of Pediatrics at the university of Helsinki already in 1957, including the post as chairman of the Children's Hospital in Helsinki, a place which he has remained faithful until this day

Niilo Hallman's contributions to our knowledge are many and important The list of his scientific publications is long and impressive

Stimulated by the academic atmosphere of Harvard University where he spent a few years early in his career, his interest was awaked for the renal problems of children and especially the complex of the nephrotic syndrome Within this field he has described a new severe but fascinating disease, the nephrotic syndrome of the newborn, particularly common in the Finnish population—the "morbus Hallman

It is further necessary to mention Niilo Hallman's numerous studies on infantile gastroenteritis, still one of the main causes of the high infant mortality in poor countries and a disease that was a great killer also in the Scandinavian countries only a few decades ago Niilo Hallman's studies in the electrolyte shift and mineral metabolism in severe diarrhoea and on the treatment of this disease have greatly increased our knowledge in this field and contributed to the considerably improved prognosis for dehydrated children Among the many other fields which Niilo Hallman has covered in his research work I would also like to mention his studies on the blood sugar of newborn children, which have helped to clarify a previously obscure area

Niilo Hallman's outstanding capacity has led him to a number of important activities in child health, pediatric research and medical science both in his own country and in international organisations Let me remind you of his leading role in the General Mannerheim League for Child Welfare, and in our sister organization, the Finnish Pediatric Association as well as his long standing contributions within the Board of the International Pediatric Association, the European Society for Pediatric Research and the European Society for Pediatric Nephrology

Yngve Larsson

Acta Pædiatr Scand 69 274–277, 1980

THE FINNISH HERITAGE OF DISEASE

NILO HALLMAN

In the early 1950 s, attention was focused on a peculiar group of neonates and young infants at the Children's Hospital of the University of Helsinki. They had progressive edema and gross proteinuria, features typical of the nephrotic syndrome (1). Only isolated cases had been previously reported in this age group, and the disease was considered very rare. These infants showed no response to the standard treatment of the nephrotic syndrome in the older child, corticosteroids and antimetabolites. Failure to thrive, with a delay especially in physical development, was the rule. All of the patients died early and most of them by the age of 2 years. The cause of death was usually an infection and not, as one might expect, renal failure with uremia.

Other characteristic features of the clinical picture included pre-term birth and low birth weight for gestation. The placenta was invariably large, with a weight more than 25% of the birth weight of the child. A cord blood sample could be obtained in some cases, and protein concentrations and electrophoretic patterns typical of the nephrotic syndrome were evident already at birth. Autopsy revealed cystic dilatations in renal tubuli. This clinical entity is congenital nephrosis (2, 3), later designated as the "Finnish type" (CNF).

Etiology of congenital nephrosis of Finnish type (CNF)

The etiology of CNF was unknown. The large placenta suggested the possibility of an immunological mechanism, but clear evidence for this hypothesis could not be found (4, 12). When autopsy samples from the kidneys were studied by microdissection, cystic dilatations of the proximal tubuli were invariably found, but these did not resemble any known cystic renal disease (11). In newborn animals, similar dilatations could be produced by injecting pregnant rats near term with aminonucleoside (14), a known nephrotoxic agent. The dilatations were explained by the filtration of protein molecules through the basement membrane and their subsequent precipitation in th[e] tubuli, causing a block in the lumen and secondary dilatation. Thus, a congenital defect i[n] renal tubuli could not be demonstrated.

On the basis of the clinical material of th[e] Children's Hospital of the University of Helsinki, consisting of about 40 families from different parts of the country, a detailed genealogical study was initiated (8). By examining hospital records and interviewing midwives about 20 additional families were found, many of which were related to previously known families.

Calculation of the proportion of affected infants from the total number of children in the families gave a result very close to 25%. There seemed to be no reason to doubt that CNF is a genetic disease, with an autosomal recessive mode of inheritance.

An analysis of the pedigrees revealed consanguinity between the parents in almost one half of the families. It came as a surprise that the relationships were rather distant and never involved a marriage between first cousins. However, such marriages were forbidden by law until 1872 and they still are rare. In addition to parental consanguinity many of the families were related, forming clusters of up to ten families especially in central, eastern and northern parts of the country. These relationships also were mostly distant, often as far as 6 to 8 generations back. This kind of cluster-

Table 1 *Finnish' recessive diseases*

Approximate number of patients known in Finland and elsewhere 1978 (10)

	Number of patients known	
	In Finland	Elsewhere
1 Aspartylglucosaminuria (AGU)	120	<20
2 Autoimmune polyendocrinopathy–candidosis–ectodermal dystrophy	40	<100
3 Choroideremia (X-linked)	60	≈200
4 Congenital chloride diarrhea (CCD)	30	30
5 Congenital nephrotic syndrome of Finnish type (CNF)	200	<200
6 Cornea plana congenita	50	<20
7 Diastrophic dwarfism	>40	≈200
8 Dystrophia retinae pigmentosa – dysacusis (DRD)	180	?
9 Hereditary fructose intolerance	20	>100
10 Hyperornithinemia with gyrate atrophy of the choroid and retina (HOGA)	30	30
11 Lysinuric protein intolerance (LPI)	30	10
12 Meckel syndrome	>30	<100
13 Metaphyseal chondrodysplasia type McKusick	>30	>80
14 Mulibrey nanism	30	5
15 Muscle eye and brain disease (MEB)	15	–
16 Neuronal ceroid lipofuscinosis		
(*a*) Infantile type (INCL)	>70	30
(*b*) Jansky Bielschowsky type	20	?
(*c*) Spielmeyer-Sjögren type	>100	?
17 Nonketotic hyperglycinemia	20	50
18 Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy	>10	>10
19 Progressive myoclonus epilepsy (PME)	>100	<100
20 Retinoschisis (X-linked)	>200	~100
21 Salla disease	30	–
22 Selective malabsorption of vitamin B_{12}	30	30

ing as well as the remoteness of both consanguinity and relationship clearly indicate that the patients come from highly inbred and isolated population groups where early sampling effect and later genetic drift have caused strong gene enrichments with an enormous risk of homozygosity

At present we know that the overall incidence of CNF in Finland is about 1 per 8000 live births and much higher in certain geographical areas We also know that the glomerular basement membrane is permeable to proteins already at an early stage of fetal development This allows the prenatal diagnosis of affected fetuses on the basis of demonstrating an elevated level of alfa fetoprotein in the amniotic fluid during the 15–16 weeks of gestation (13) In at least 60–70% of such cases, alfafetoprotein concentration is high also in maternal serum in the first trimester The basic defect of CNF is still unknown

Recessive diseases in Finland

After the elucidation of the clinical picture and genetic nature of CNF, several other single-gene defects, most of them autosomal recessive, have been detected and found to be particularly prevalent in the Finnish population The number of cases diagnosed in Finland is usually larger than that in the rest of the world combined (Table 1) Naturally, these figures are far from accurate but they do reflect true differences in the incidence and prevalence Thus, there are grounds for using the term "*the Finnish heritage of disease*"

The diseases listed cover a wide clinical spectrum (6, 10) Many are associated with disturbances of growth and development, neurological symptoms including visual and hearing loss, or unfortunately, progressive mental retardation Some can be diagnosed at birth or at an early age, particularly if the possibility is kept in mind, others manifest themselves only in adulthood A detailed discussion of the individual diseases at this time is clearly impossible However, it is important to realize that such presumably rare diseases do occur in the Finnish population At present, with heavy immigration of young families from Finland into Scandinavia, particularly Sweden, the Swedish medical community should also be aware of the peculiar pattern of genetic disease among Finns Specific diagnosis is important not only from the strictly therapeutic point of view but also for genetic counseling

In the spectrum of genetic disease in Finland, a number of recessive disorders have an incidence similar to that in the other European

countries. However, other well-known metabolic diseases, such as galactosemia, hepatic glycogenosis, cystinosis and maple syrup urine disease, seem to be absent in the Finnish population. The most striking example is phenylketonuria (15), which is considered the most common preventable cause of mental retardation. In a careful search through all the mental institutions in Finland, only four cases were detected, and a pilot screening study of 80 000 newborn infants with the Guthrie test gave some false positive results but not a single case of phelyketonuria. Therefore, neonatal screening for phenylketonuria has not been implemented in Finland. With regard to the "Finnish diseases", either indications or methods for neonatal screening are lacking.

Thus, in addition to the enrichment of certain rare genetic diseases in the Finnish population, our disease heritage is characterized by the absence of other disorders that are prevalent in other populations.

Population history of Finland

Our information about the origin of the Finns as a people is based on archeological and linguistic studies (5, 7). Iron Age burial grounds have been found in only a few locations in western and southwestern Finland, and the country seems to have been largely uninhabited at that time. The origin of the Lapps is not known with certainty, but they are genetically closely related to the Finns, who gradually pushed them northwards. The number of the Lappish population has probably always been relatively small.

The ancestors of the present-day Finns have migrated into the country from the south and southeast, starting during the early centuries after Christ. The populations of Finland, Carelia, and the Baltic countries still constitute a rather homogenous linguistic group. During our history as part of Sweden, some migration from Sweden into Finland also took place mainly into the southwestern areas, Åland islands, and the coast of the Gulf of Bothnia.

Despite the fact that Finland belonged to the kingdom of Sweden until the year 1809 and thereafter to czarist Russia until 1917 the country has never been occupied except for brief periods during wars. Throughout recorded history, the Finnish population has been effectively isolated from outside genetic influences. This is explained in part by geographical factors, in part by the location between two countries with different heritage and language. This accounts for a specific assortment of recessive genes.

The migration of the ancestors apparently took place gradually and in small groups. The immigrants then slowly drifted northwards first as hunters and fishers, later setting up villages and starting agriculture. Vast forests and innumerable lakes have acted as barriers between new settlements, which thus developed into genetic isolates. Studies by Nevanlinna (5) have indicated that up until fairly recent times it was customary even in southern Finland to marry from one's own village or commune rather than go elsewhere.

In part because of the small number of original settlers, the total population of the country has increased slowly. Contributing factors to this have been frequent wars, epidemics and famines. After the great famine during the early years of the 18th century, the population numbered only about 400 000. By 1850, it had increased fourfold and at the turn of the century reached 2.6 million. If the present trend in birth rate continues, the population of Finland will start to diminish before reaching 5 million. Another recent development is rapid urbanization: in 1930 80% of the population lived in rural areas, now less than 50%. Since migration has mainly been directed into towns rather than between rural areas, the genetic isolates have largely been maintained, although with diminished population.

The circumstance I have described provide an explanation for the Finnish heritage of disease. In such circumstance, founder effect and genetic drift readily account for the regional clustering of some rare genes and thus

for the frequent occurrence of homozygotes with a rare recessive disorder. The small number of original ancestors of the Finns has created a specific assortment of rare genes The continuing isolation has maintained this assortment and chance has further accentuated it by enriching some genes and eradicating others As expressed by Norio et al. (9) the rare flora has a rare soil to grow

Interesting observations can be made on the geographic distribution of certain of the diseases I have mentioned by plotting the birthplaces of the parents and grandparents of patients on the map of Finland CNF originates in central Finland and southern Ostrobothnia, where the settlement of the country stopped for a time, before again starting to spread northwards during the 17th and 18th centuries Mulibrey nanism is enriched in the lake district of central and eastern Finland, while congenital chloride diarrhea is clearly concentrated in more eastern areas The most restricted geographic distribution is shown by retinoschisis in southwestern Finland Similar maps could be presented of many other "Finnish" diseases Despite the fact that the southern areas were the first to be settled and have been most densely populated, very few rare recessive diseases are encountered there This is explained by more frequent contact and intermingling both within the area and the outside world, preventing the formation of genetic isolates

During the past decades, living conditions and population structure in Finland have changed rapidly Yet some degree of regional isolation has been maintained Nevanlinna (5) estimates that even today the parents of about one-third of all infants are from the same or very closely located area Therefore, pediatricians in Finland will probably continue to see many of the rarities I have described

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PROMOTION OF BREAST FEEDING. EFFECT ON NEONATES OF CHANGE OF FEEDING ROUTINE AT A MATERNITY UNIT

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ABSTRACT. Verronen, P , Visakorpi, J. K , Lammi, A , Saarikoski, S. and Tamminen, T. (Department of Clinical Sciences, University of Tampere, and Department of Obstetrics and Gynaecology, Central Hospital of Tampere, Finland) Promotion of breast feeding: effect on neonates of change in feeding routine at a maternity unit. Acta Paediatr Scand, 69. 279, 1980.—The effect on the health of neonates of a change in neonatal routine care, including general rooming-in, breast feeding on demand and avoidance of supplementary bottle feedings was studied in conjunction to a breast feeding campaign at a maternity unit. There was an accentuated weight loss in the neonatal period during ad libitum breast feeding The mean serum bilirubin of clinically jaundiced infants was slightly higher on a 4 hourly feeding schedule with supplementary bottles than on the new regimen There was a [illegible] supplementary bottles and were excluded from the study.

KEY WORDS Breast feeding, hyperbilirubinemia, hypoglycaemia

Breast feeding has become increasingly popular in recent years, and various programmes have been created in order to promote it Changes in the routine of infant feeding during the neonatal period, including rooming in, on demand-feeding and avoidance of supplementary formula feedings have been recommended as part of such programmes (1). It is, however, possible that these changes in routine neonatal care might have certain effects on the health of newborns These thoughts came to our mind as a breast feeding promotion programme was conducted at our maternity unit. It was therefore decided to study this aspect. We determined the effect of the programme on the amount of weight loss in the neonatal period and on the incidence of neonatal jaundice and spontaneous hypoglycaemia.

MATERIAL AND METHODS

Breast feeding promotion programme At the Central Hospital of Tampere, changes were made in the routine care of newborns, starting in October 1978, according to Table 1 A group of mothers and children has been followed at two child health care centers and the promotion programme has already now proven successful 85% of the study mothers are breast feedings at three months, 75% without supplements Comparative control figures at the same child health care centers 6 months earlier were 65 and 58%

Study group Infants born in January and March 1979 were selected as the study group Infants born during the same months one year earlier formed a control group Those infants fed differently because of risk for hypoglycaemia were excluded (Table 2) A risk factor was considered present in the child weighed less than 2 500 grams or over 4 500 grams, was small for-dates or large for-dates, had an Apgar score less than 7 at either 1 or 5 minutes or if there was maternal diabetes, latent diabetes or severe toxaemia Another group of infants was transferred to the children s ward because of sickness and accordingly fed differently and excluded from the study, except from the study concerning hypoglycaemia The records of these children were viewed in order to find any cases whose symptoms might be due to insufficient milk intake at the breast

The study group and the control group were similar regarding the amount of risk infants and sick infants who were excluded from the study (Table 2) The obstetrical management of deliveries was also closely similar except for some minor differences (Table 3)

Table 1 *Breast feeding promotion programme*

	Earlier routine	Promotion programme
Mode of mother–child interaction in delivery room	Early nursing	Skin to skin contact early nursing
Rooming in	Voluntary 3rd day after delivery	Routine 12 hours after delivery
Breast feeding routine		
Schedule during day	4 hourly (5 times)	On demand (6–7 times) except during first 12 hours when fed 4-hourly
Night feedings	Bottle once	Breast once
Technique	1 breast at a time	Both breasts
Manual expression	Always after feeding	When needed
Supplementary feedings (bottle or spoon)	When needed	Avoided 1/3 received mostly temporarily
Amount of formula ordered by 3 maternity wards/month	150 liters	18 liters
Instruction of mothers	Not planned	Printed sheet

Assessment of weights The infants were weighed naked in the morning before feeding on the third and the fifth day after delivery

Assessment of jaundice Serum bilirubin was determined if the infant was considered clinically jaundiced Total serum bilirubin was measured from heel stick blood by an American Optical Bilirubinometer The peak serum bilirubin concentration during hospital stay was recorded

suggestive of hypoglycaemia A diagnosis of spontaneous hypoglycaemia was made if two blood glucose values were less than 1 7 mmol/l or if there was one such value while the infant had clinical signs of hypoglycaemia (tremor pallor flaccidity jitteriness convulsions)

Calculations The results were calculated by computer and significances of differences between study and control groups were determined by Student s *t* test

RESULTS

Weight loss Infants lost significantly more weight in the study group, both on the third and the fifth day (Table 4)

Jaundice More infants had their bilirubi determined in the study group (Table 5) Ther was a slightly lower level of mean peak bil rubin values in the breast feeding promotio group There was a high percentage of signif cant hyperbilirubinemia, defined as a peak bil rubin >205 µmol/l, in both groups Children c those mothers who received oxytocin infu sions during labour were equally jaundice compared to the rest

Incidence of spontaneous hypoglycaemia There was no significant difference in the in cidence of spontaneous unexpected hypo glycaemia between study and control groups In this study also the infants transferred to th children s ward were included The incidenc was 1 0% in the study group and 1 1% in th control group

Other conditions possibly related to low food intake Among children admitted fron

Table 2 *Groups studied*

	Control group	Study group
No of infants born	707	689
No of infants excluded		
Risk for hypoglycaemia	96	111
Sick transferred	28	20
No data available	7	7
No of infants studied	574	551

Table 3 *Obstetrical management of deliveries*

	Control group	Study group
Caesarean sections %	5 0	5 6
Oxytocin infusions %	48	44
Epidural analgesias %[a]	0	2 1
Length of stay in hospital days	6 4	6 0

[a] For caesarean section only

Table 4 *Weight loss*

	Control group	Study group
Mean birth weight g	3 565	3 542
Mean weight loss 3rd day %	5 6*	6 3
Mean weight loss 5th day, %	4 5*	5.3

* $p<0\ 001$

Table 5 *Jaundice*

	Control group	Study group
Clinical jaundice %	51	59
Mean total bilirubin μmol/l	224*	210
Mean total bilirubin >205 μmol/l %	33	32
Treated by phototherapy %	10	11

* $p<0\ 01$

the newborn nursery to the children's ward there was one full term infant in the study group who has lost 13% of his birth weight by the 5th day and was transferred because of lethargy and poor feeding His condition was suspected to result from prolonged insufficient milk intake at the breast, because no other explanation could be found

DISCUSSION

The general decrease in the incidence of breast feeding in the past few decades might partly be due to the general trend of centralizing deliveries to big hospitals which may lack the facilities for rooming in and often practice rigid feeding schedules with ample supplementary feedings to the newborns It has been demonstrated that breast feeding can be promoted by introducing rooming in (7) and by practicing self-demand feeding in the newborn period (6) Thus, if breast feeding is to be promoted in a community, certain changes in the routine care of infants at the maternity wards are often necessary It is, however, important to bear in mind that these changes can affect the well being of neonates Their nutritional status can be evaluated by the amount of weight loss and the incidence of jaundice and hypoglycaemia can reflect possible dangers to the newborns caused by a breast feeding promotion programme which includes avoidance of supplementary bottle feedings

Regarding weight loss, this study supports the results of Dahms et al (3), who noted a significantly greater weight loss in the neonatal period among children breast fed on demand compared to those breast fed 4-hourly with supplements Dahms et al (3) did not find significant differences in the incidence of jaundice The drop in peak serum bilirubin noted in this study in the ad libitum breast fed group might be attributed to some other factor besides feeding or it might be purely incidental

The 32–33% incidence in both study and control groups of significant hyperbilirubinaemia, defined as peak serum bilirubin >205 μmol/l was considerably higher than in other recent surveys (2, 5, 10) The reason for the high figures in this study is not clear Oxytocin infusions during labour have been noted to increase jaundice (4), especially when used to induce labour (8) In our series there was a high (44–48%) rate of oxytocin infusions with an approximate ratio of 1 2 for induction/augmentation of labour However, infants whose mothers received oxytocin were equally jaundiced compared to the rest

The reason behind the wide practice of giving supplementary bottles to full term breast fed infants is not quite clear The findings by Smallpeice & Davies (10) that premature babies fed early with liberal amounts of breast milk developed lower bilirubin values and less hypoglycaemia and regained their birthweight sooner caused a clear change in the feeding routine of prematures The effect of this change might have radiated further to influence also the feeding of full term infants

As a conclusion, the practice of ad libitum breast feeding and avoidance of supplementary bottle feedings in the neonatal period as a part of a breast feeding campaign did not

seem harmful to the health of the neonates. There was no increase in the incidence of jaundice or spontaneous hypoglycaemia or any other harmful effects in spite of a slower increase in weight It is, however, important to bear in mind that from this study all those infants who were at risk for hypoglycaemia were excluded and given supplementary feedings.

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Acta Pædiatr Scand 69, 283–286, 1980

PREVENTION OF CEREBRAL PALSY IN MOTOR RISK INFANTS BY TREATMENT *AD MODUM* VOJTA

A Controlled Study

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ABSTRACT. Brandt, S., Lønstrup, H , Marner, T., Rump, K. J , Selmar, P., Schack, L. K (Clinic for Cerebral Palsy and Child Neurology, Orthopedic Hospital, Rigshospitalet, Copenhagen, Denmark) and d'Avignon, M., Noren, L. and Årman, T. (The Paediatric Clinics of St Goran's and Danderyd Hospitals, Stockholm, Sweden) Prevention of cerebral palsy in motor risk infants by treatment ad modum Vojta. Acta Paediatr Scand, 69, 283, 1980.—The proposal by V. Vojta in 1974 to prevent development of cerebral palsy in 'motor risk' infants by special treatment has been investigated in 11 Danish and 10 Swedish babies and compared with 30 control infants with a similar risk, who were not given Vojta treatment. We found a tendency for 'uncomplicated' cerebral palsy cases to accumulate in the control group, although the difference was non-significant on a 5% level. Further controlled studies must be completed before it is possible to accept the prophylactive treatment of cerebral palsy recommended by Vojta.

KEY WORDS Cerebral palsy, Vojta treatment, risk babies

Vojta, in an extensive monograph (10), recommended systematic exercises (reflex creeping and reflex turning) in prone so-called motor risk infants, diagnosed according to a series of 10 standardized reflexes of posture, combined with other possible deviations of tonus, either uni- or bilateral. He claims that cerebral palsy (c p) can be successfully prevented by his treatment, when given daily by one of the parents for at least 6 months in infants in whom generalized and diffuse cerebral atrophy leading to severe mental defect or other serious complications of the motor disturbance is not to be expected

Adaptive and reparative mechanisms, resulting in an often surprisingly normal motor development in infants who had even alarming neurological symptoms in the newborn period, are known to child neurologists and must be considered to make a major contribution to a successful motor development (4, 5, 9).

Vojta seems well aware of this obstacle to any obvious proof of his theories. Being himself emotionally convinced about the correctness of his ideas, he has refused to use acceptable controlled studies to document his thesis. His endeavours to present some documentation, directly or 'indirectly', based on his own material of apostate cases or by comparison with various publications concerning the outcome of risk babies with various symptoms are unconvincing (2, 3, 6) He also feels that his procedure is superior to other recommended systems of very early treatment of c p., such as that of Bobath (1). His reasons for desisting from a controlled study are of an ethical nature He would suffer from remorse on withholding his treatment from an infant whom he diagnosed as being at risk for c p. In the second edition (11) of his book he seeks further support for his view.

PRESENT STUDIES

Since 1975 we have tried to compare results of Vojta s procedures, diagnostic as well at therapeutic, with the development of similarly selected non-treated infants,

or—in some cases—infants given other treatment mostly for the benefit and comfort of anxious parents who could hardly be expected to accept a trial in which no treatment at all was given to every second child Usually alternative treatment was given according to the principles recommended by B Bobath (1) One of us (L K S) a physiotherapist spent several periods at Vojta's clinic in Munich to familiarize herself with his technique

Selection of material

We cooperated with three pædiatric departments in the Copenhagen area receiving newborns for intensive supervision and follow up (the neonatal ward of Rigshospitalet, the paediatric hospital at Fuglebakken and the pædiatric department of the county hospital in Glostrup)

Our colleagues were instructed and supplied with detailed information about the normal and possible abnormal neurological findings at the age of 5 months including 7 postural reflexes as recommended by Vojta (see 8 details to be found in 10) Only infants between 4 and 6 month of age were selected irrespective of their birth weight Babies with abnormal findings were referred to our clinic where the testing was repeated We selected for our study only infants in whom at least four abnormal reflexes were found This was in accordance with the conditions recommended by Vojta in 1974 A total of 34 babies were found acceptable for scientific study (3 in 1974 18 in 1975 11 in 1976 and—surprisingly—only 2 in 1977)

A study of the birth histories of these 34 infants provided us with good evidence of brain insults 21 had a birth weight under 2 500 g and in 23 clinical signs of perinatal anoxia had been noted

Method

The 34 babies were separated by lot into two groups one Vojta group comprising 15 babies between 4 and 6½ months of age and a control group of 19 babies (aged 4 to 7 months)

Each Vojta baby was treated at home by one relative after repeated instructions by one of us (L S K) (7) All babies were re examined every 3 months by members of the medical and physiotherapy staff The senior author (S B) strove to remain unaware of which group each baby belonged to Not until 1978 did he assemble the results before opening the key to the ballot

RESULTS

Our findings are presented in Tables 1 and 2

Cases withdrawn from the study

One control baby died at the age of 6 months from asthma One boy in the Vojta group had already symptoms of c p before treatment was started and had been included because Vojta maintains that treatment might even benefit infants with early signs of c p He did not improve and was withdrawn from the group and given other treatment Three babies left the study because their mothers suffered too much from the intense screaming of their babies According to Vojta this is not due to pain but to the intense struggle by the infant against the fixation in prone posture necessary to elicit the righting reflexes, which are intended to bring the baby up into a creeping position Two of these infants were followed up in 1978 by S B One was normal at the age of 3½ years, the other when he was 3 years old

Table 1 *Development in 34 cerebral palsy risk babies, given (V group) or not given (C-group) preventive treatment by V Vojta's method*

	V group	C group
Normal development	8	5
Normal development after other type of treatment		7
C P in spite of treatment	3	4
C P (no treatment)		2
Left the study	4	
Died		1
Total	15	19

Fate of remaining cases included in the study

In the Vojta group 8 had developed normally Three became handicapped by complicated c p (1) a 19 month old infant was microcephalic with severe tetraplegia, kyphosis on sitting, rigid limbs, hyperreflexia and—probably low grade—mental deficiency, (2) a 2 year old boy, also microcephalic, was an imbecile, with severe motor rigidity, (3) one boy had already developed a severe spastic dystonic tetraplegia by the time he was 1½ years old None of the babies in the Vojta group developed non complicated spastic hemi para or tetraplegia

In the control group 12 had developed normally, 6 had c p (1) one with severe rigid dystonic tetraplegia, probably with mental subnormality (1½ years old), (2) a 2½ year-old boy had a severe, probably complicated c p,

Table 2 *Final results in combined Danish (D) and Swedish (S) series of motor risk babies given (V group) or not given (C group) CP preventive treatment a m Vojta*

	V group			C group			Total
	D	S	D+S	D	S	D+S	
Normal	8	8	16	12	3	15	31
Uncomplicated CP	0	1	1	4	3	7	8
Complicated CP	3	1	4	2	5	8	12
All with CP	3	2	5	6	9	15	20
All evaluated	11	10	21	18	12	30	51

(3) one was left with a mild inferior monoplegia (2½ years old), (4) one had a moderate spastic tetraplegia at the age of 4 years, (5) one a second grade spastic tetraplegia, and (6) one boy had obvious symptoms from the age of 7 months of a moderate spastic tetraplegia Vojta would—we feel—have classified 3 or 4 of these babies as neglected cases in whom his treatment would have been able to prevent c p

The given treatment was in every case continued until no neurological symptoms could be registered at the regular follow ups If symptoms persisted after the age of one year we tried supplementation with other types of treatment The total number of c p cases in the Vojta and control group was equal (3 out of 11 and 6 out of 18 respectively (Table 2)

Since Vojta s latest conclusion (11) is that his treatment prevents only non-complicated c p and will fail in complicated cases due to extensive brain lesions we compared the non complicated cases of c p in the two groups The apparent difference (0 cases in the Vojta group of 11 and 4 out of 18 in the controls) was non significant at a 5% level (cand act J Nyboe)

DISCUSSION AND SUPPLEMENTARY DATA

We were disappointed to find that only 2 infants had been referred to us during 1977 in contrast to 29 during the preceding 3 years According to our suppliers of case material, this could not be explained by the declining interest in our project, but might possibly reflect an improvement in obstetrics and neonatal care

In our strivings, therefore, to increase our figures we contacted a group of pædiatric colleagues in Sweden, whom we knew were carrying out a similar study The only difference was that the Swedish team had required 5 to 6 abnormal postural reflexes before they included a baby in the study (which is a stricter rule than given by Vojta in 1974), and they had tried to correct for prematurity by selecting infants who were x months old + the number of months they were born before term The Swedish team had run into the same difficulties as we had in trying to collect a material large enough for statistical analysis and were therefore inclined to abandon their project, since their statistical consultant had judged that at least 35 individuals in eac group would be necessary The combined figures from our Danish and the Swedish studies are shown in Table 2 Five out of 21 babies in the Vojta group and 15 out of 30 controls had c p The apparent difference in favour of the Vojta treated group was still non significant at a 5% level If we compare the infants with uncomplicated c p in the combined material, we find only one out of 21 in the Vojta group in contrast to 7 out of 30 in the control group Although from a statistical point of view these figures show no significant difference at the 5% level, it may be permissible to talk about a certain *tendency* in favour of the Vojta treat-

ment, a conclusion which became accepted by our statistician

Final conclusions

Although impossible to prove statistically, our studies from Denmark and Sweden made it permissible to conclude that the Vojta treatment might prevent the development of un complicated c p , whereas (complicated) sequelae due to extensive brain lesions were inevitable

ACKNOWLEDGEMENT

The Danish participants in this study wish to express their gratitude to the staffs of the hospitals who provided us with the Danish material to our Swedish colleagues for lending us their case material and to cand act J Nyboe for his assistance with the statistical calculations

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Submitted July 17 1979
Accepted Oct 16 1979

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Acta Pædiatr Scand 69 287–292, 1980

SERUM DEHYDROEPIANDROSTERONE (DHA) AND SULPHATE (DHAS) AFTER ACUTE GROWTH HORMONE THERAPY

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ABSTRACT Rudd, B T, Rayner, P H W, Bassett, R M and Williams, J W (Institute of Child Health, University of Birmingham and Department of Clinical Endocrinology, Central Birmingham Health District, England) Serum dehydroepiandrosterone (DHA) and sulphate (DHAS) after acute growth hormone therapy Acta Paediatr Scand, 69 287, 1980 —To test the hypothesis that growth hormone (hGH) may increase adrenal androgen production dehydroepiandrosterone (DHA) and its sulphate (DHAS) concentrations were measured by radioimmunoassay in the serum from 7 children with growth hormone deficiency, 2 of whom had delayed puberty Two injections of hGH (10 mg) were given 48 h apart and the hormone concentrations measured at 3, 6, 24 and 48 h after the first injection, 3, 6 and 24 h after the second Basal DHA levels were positively correlated with age and bone age in 6 of the 7 patients ($p<0.05$) Increments of DHA and DHAS above or below basal at each [illegible]

in DHA during the first and second 24 h of the test ($p<0.05$) DHAS concentrations showed little change throughout the test for all children It is suggested that some children with growth hormone deficiency and receptive adrenals, increase their serum DHA concentrations after acute hGH therapy

KEY WORDS hGH deficiency, delayed puberty, acute hGH therapy, adrenal androgens

Dehydroepiandrosterone (DHA) and its sulphate (DHAS) are androgens secreted and synthesized by the adrenal (5, 9, 15, 17, 25, 33) On the basis of a model with two pools, one of DHAS and one of DHA, Vande Wiele et al (31) have calculated secretion rates of DHA and DHAS and peripheral interconversion of DHA to DHAS and DHAS to DHA The mechanisms which control the secretion and interconversion of these two steroids are not understood

ACTH when administered intravenously, can increase circulating levels of DHA and DHAS within 30 min (24) However, there is so far no definitive evidence that the circulating concentration of endogenously produced ACTH rises during normal development

Serum concentrations of DHA and DHAS are low prepubertally, then rise progressively throughout puberty, earlier in girls than in boys (8, 13, 18) The rise has been cited as evidence that luteinizing hormone (LH) which also increases at this time, is the trophic hormone which stimulates adrenal androgen production as first suggested by Albright (1) Mills et al (21) reported that patients with clinical evidence of an enhanced production of LH when given ACTH, show an increased excretion of 17 oxosteroids, which are in part metabolites of DHA and DHAS

Prolactin (hPrl) has been favoured as another pituitary hormone which may increase adrenal androgen biosynthesis Increased concentrations of serum prolactin are frequently accompanied by a high concentration of DHA and DHAS in the serum of female patients with hyperprolactinaemia (4, 10, 32) Conversely, an increased concentration of hPrl in the

ment, a conclusion which became accepted by our statistician.

Final conclusions

Although impossible to prove statistically, our studies from Denmark and Sweden made it permissible to conclude that the Vojta-treatment might prevent the development of uncomplicated c p., whereas (complicated) sequelae due to extensive brain lesions were inevitable.

ACKNOWLEDGEMENT

The Danish participants in this study wish to express their gratitude to the staffs of the hospitals who provided us with the Danish material, to our Swedish colleagues for lending us their case material and to cand act J Nyboe for his assistance with the statistical calculations

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Submitted July 17, 1979
Accepted Oct 16, 1979

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Acta Pædiatr Scand 69 287–292, 1980

SERUM DEHYDROEPIANDROSTERONE (DHA) AND SULPHATE (DHAS) AFTER ACUTE GROWTH HORMONE THERAPY

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ABSTRACT Rudd, B T, Rayner, P H W, Bassett, R M and Williams, J W (Institute of Child Health, University of Birmingham and Department of Clinical Endocrinology, Central Birmingham Health District, England) Serum dehydroepiandrosterone (DHA) and sulphate (DHAS) after acute growth hormone therapy Acta Paediatr Scand, 69 287, 1980 —To test the hypothesis that growth hormone (hGH) may increase adrenal androgen production dehydroepiandrosterone (DHA) and its sulphate (DHAS) concentrations were measured by radioimmunoassay in the serum from 7 children with growth hormone deficiency, 2 of whom had delayed puberty Two injections of hGH (10 mg) were given 48 h apart and the hormone concentrations measured at 3, 6, 24 and 48 h after the first injection, 3, 6 and 24 h after the second Basal DHA levels were positively correlated with age and bone age in 6 of the 7 patients ($p<0.05$) Increments of DHA and DHAS above or below basal at each time interval were calculated The mean increments were higher ($p<0.01$–0.05) at 3 h after the first injection and at 24 h ($p<0.025$–0.05) after the second when compared to any other

growth hormone deficiency and receptive adrenals, increase their serum DHA concentrations after acute hGH therapy

KEY WORDS hGH deficiency, delayed puberty, acute hGH therapy, adrenal androgens

Dehydroepiandrosterone (DHA) and its sulphate (DHAS) are androgens secreted and synthesized by the adrenal (5 9 15, 17, 25 33) On the basis of a model with two pools, one of DHAS and one of DHA Vande Wiele et al (31) have calculated secretion rates of DHA and DHAS and peripheral interconversion of DHA to DHAS and DHAS to DHA The mechanisms which control the secretion and interconversion of these two steroids are not understood

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Table 1. *Patient endocrine status before the test*

Pat no	Age	Sex	Pubertal stage (Tanner)[a]	Bone age[b] (y)	Weight (kg)	Growth velocity (cm/y)	hGH and cortisol maximum response to insulin induced hypoglycaemia[c]: hGH (mμ/l)	Cortisol (nmol/l)	T4/PBI[d] (nmol/l)	Diagnosis
1	12 25	F	I	10 4	24 4	3 2	3	642	100[e]	hGH deficiency
2	19 5	M	I	13 5	41 2	2 3	4	603	94	hGH deficiency a delayed puberty
3	18 5	M	II	12 2	52 8	3 5	2	624	117	hGH deficiency a delayed puberty
4	12 0	M	I	7 6	28 3	4 4	10	918	79[e]	hGH deficiency
5	9 67	M	I	6 5	17 2	2 5	7	452	98	hGH deficiency a emotional depri syndrome
6	9 0	F	I	3 9	15 5	2 9	1	662	68	hGH deficiency
7	11 0	M	I	9 5	19 7	3 9	3	708	122	hGH deficiency

[a] Tanner (29)
[b] Tanner et al (30)
[c] 0 1 μ insulin/kg A normal response is a concentration of hGH >20 mμ/l and for cortisol, >280 nmol/l
[d] Normal reference values 60–135 nmol/l
[e] PBI

serum of some female patients with hyperprolactinaemia does not always lead to raised basal levels of DHA or its sulphate (16, 22) In children approaching puberty, the rise in radioimmunoassayable hPrl appears to be confined to girls (26) and approximates to the time when serum DHA concentrations increase (13)

Evidence has been presented which suggests that growth hormone may also influence the interconversion or secretion of DH/ DHAS The studies of Burstein (6) shown an increase in steroid sulphatase : ity when bovine growth hormone is giv rats and this change in sulphatase activit increase the conversion of DHAS to I The biosynthesis of radio-labelled DHA its sulphate is increased when ^{14}C lat acetate and hGH are added to human f adrenals *in vitro* (14) The investiga

Table 2 *DHAS (μmol/l) and DHA (nmol/l) concentrations during the test*

h from basal	0	3	6	24	48	51
DHAS mean	1 90	1 87	1 83	1 60	1 63	1 73
±S E M	0 43	0 45	0 43	0 35	0 37	0 46
Range	0 5–3 8	0 4–3 7	0 3–3 4	0 4–2 9	0 3–2 8	0 2–3
Mean increments		−0 03	−0 07	−0 3		+0 10
±S E M		0 079	0 10	0 10		0 10
DHA mean	6 40	9 42[a]	5 54	5 84	6 46	5 81
±S E M	2 04	3 34	2 03	2 45	2 02	2 31
Range	1 5–17 0	0 7–23 0	0 7–16 0	0 5–19 0	0 5–17 0	0 5–18
Mean increments		+2 22[b]	−0 86	−0 49		0 00
±S E M		1 35	0 35	0 49		0 23

[a] Six observations only
[b] Significantly higher than at any other time 3 h vs 6 h $p<0.01$, 3 h vs 24 h $p<0.05$ 72 h vs 51 h $p<0.025$ 54 h $p<0.05$ Data not normally distributed

quoted suggest that growth hormone may *affect steroid sulphatase* activity and adrenal androgen synthesis

In the present report, an attempt has been made to test the hypothesis that acute hGH *therapy* when given to children with different degrees of growth retardation and bone maturation can increase blood concentrations of DHA

MATERIALS AND METHODS

Patients

Seven patients entered the study 2 females and 5 males with an age range 9–19 5 years All seven had isolated growth hormone deficiency and two of the males had associated delayed pubertal development Table 1 documents their clinical and endocrine status prior to the study

Test protocol

Informed parental consent was obtained before the children entered the study All patients were hospitalised A baseline serum sample was obtained after an overnight fast on day one then intramuscular injection of hGH was given (Wilhelmi 6 patients Rabin preparation 1 patient) Further samples were collected at 3 6 24 and a second basal sample collected at 48 h after the injection followed by a second 10 mg injection of hGH and blood samples at 3 6 and 24 h All the children proceeded onto long term growth hormone therapy on completion of the test

Growth hormone levels were measured by a modification of the double antibody radioimmunoassay technique as previously described (12) using the W H O 1st International Reference Preparation 66/217 as standard Cross reactivity of the hGH antibody with hPrl was <0 1%

54	72
1 73 0 43	1 66 0 37
0 3–3 4	0 6–3 0
0 10 0 048	+0 03 0 049
6 36 3 04	8 67 3 73
0 6–23 0	1 0–29 0
0 54 1 00	+2 86 1 49

The Wilhelmi hGH used for injection was randomly checked *for hPrl content* by radioimmunoassay (11) No significant activity was detected DHAS was measured by radioimmunoassay as described by Smith et al (27) DHA was measured by radioimmunoassay with the same *antibody as used for DHAS* (2 3)

Statistical analysis

Patient data was tested for homogeneity (Bartlett s Test) before parametric statistics were applied and when the data was not normally distributed tests of significance between hormone concentrations at different times were made using the Mann Whitney Test Linear regression equations were calculated by standard statistical procedures (28) Increments for both DHA and DHAS were calculated by summation of the difference between individual basal concentrations and the concentrations for each time interval after the first and second injections of hGH

RESULTS

Basal levels

DHAS basal concentrations before the first injection in the seven patients, ranged from 0 5–3 8 μmol/l which were similar to values found for normal children aged 10–15 years of 0 3–3 9 μmol/l The two eldest boys (nos 2 and 3) with delayed puberty, had concentrations of DHAS of 2 7 and 1 9 μmol/l respectively which were within the range for prepubertal children and at the lower limits of normal for *males and females* in the age range 18–30 years *(2 6–12 3 μmol/l) DHA concentrations* ranged from 1 5–17 0 nmol/l compared to values of 1 0–11 2 nmol/l found for children aged 6–15 years without endocrine disease The highest concentration (17 0 nmol/l) was found in the female patient no 1

Basal DHAS and DHA before hGH concentrations rose commensurate with age and bone age in all but patient no 1 The basal DHA concentration in this patient was outside +2 S D of the mean for the *remaining six* patients The correlations, age DHA and bone age DHA for the six patients, were $r=0\ 66$ ($p<0\ 05$) and $r=0\ 64$ ($r<0\ 05$) respectively The corresponding correlations of age or bone age with DHAS *failed to reach significance* ($p>0\ 05$)

Table 2 documents the mean (±S E M) and ranges for DHAS and DHA during the course

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Mean increments ±S E M		0 03 0 079	−0 07 0 10	−0 3 0 10		+0 10 0 10
DHA mean ±S E M	6 40 2 04	9 42[a] 3 34	5 54 2 03	5 84 2 45	6 46 2 02	5 81 2 31
Range	1 5–17 0	0 7–23 0	0 7–16 0	0 5–19 0	0 5–17 0	0 5–18 0
Mean increments ±S E M		+2 22[b] 1 35	−0 86 0 35	−0 49 0 49		0 00 0 23

[a] Six observations only
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$p<0 05$ Data not normally distributed

that could be ascertained was a mean age and bone age difference between the patients (Table 1) As a point of interest, the patient (no 1) with the highest basal and increment of DHA was a female and she had a more ad vanced age and bone age than the other girl in the study

The variable responses to hGH are unlikely to be related to differences in the release of endogenous ACTH because an adequate corti sol response was obtained in all children after insulin induced hypoglycaemia The degree of stress produced after intramuscular injections of hGH would also be similar for all the chil dren

It is unlikely that the differences in patient response are related to the diurnal rhythm of DHA because the samples were taken at ident ical times of day for each patient and to our knowledge no major changes in DHA occur between days during daylight hours

It was not possible to demonstrate a signif icantly different fall in DHAS in those children who increased their DHA levels when com pared to those who did not Had this been so, an explanation for the difference between those patients whose DHA rose and those whose DHA did not would be their ability to convert DHAS to DHA after induction of ste roid sulphatase activity by hGH This mechan ism of action of hGH cannot entirely be ruled out because the half life of labelled DHAS (*T* ½ *8–11 h*) compared to 25 min for DHA (5), and the thousand fold higher concentrations present in serum compared to DHA, would make it extremely difficult with the present protocol to demonstrate a significant fall in DHAS

Added to these difficulties are the observa tions of Linderkamp et al (19) who have shown an increase in blood and plasma volume and red cell mass after prolonged hGH admin- istration to growth hormone deficient children Thus if the plasma volume should also in crease after acute hGH therapy a small but significant decrease in DHAS concentration would be difficult to detect

Pre priming of the adrenal by prolactin over an extended period may also be required be fore an adrenal androgen response to hGH is elicited Metabolic changes attributable to pro- lactin, do occur in steroid producing cells (23) Further, specific binding of ^{125}I labelled pro lactin and hGH to adrenals from rats has been demonstrated (7, 20) Data from the human is however, lacking

The study presented suggests that there is a critical time in the maturation of patients with hGH deficiency and before clinical signs of puberty, when the adrenal cortex is responsive to acute hGH as reflected by increased cir- culating concentrations of DHA This respon- siveness of the adrenal to exogenous hGH may prove to be a useful guide as to whether or not growth hormone deficient children of ap- propriate age or bone age will proceed through an adrenarche to full pubertal development

ACKNOWLEDGEMENTS

The authors are grateful to Professor W R Butt for his encouragement and facilities he provided the Medical Research Council for generous supplies of hGH and the nursing staff of the Children s Hospital for their excellent supervision of the children during the studies

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of the test Mean increments (above or below) basal mean before the first injection and again after the second, were recorded to take account of rising basal concentrations with age or bone age As there was no statistical difference between the mean basal concentrations of each steroid before hGH when compared to 48 h after the first injection, increments for the two 24 h periods were calculated separately using the basal values before hGH and those at 48 h after the first injection At 24 h after the first and second injection of hGH, the mean DHAS was lower than but not significantly different from pre treatment mean

The mean DHAS increment fell to a minimum at 24 h after the first injection, but the fall was statistically unrelated to the changes in DHA concentrations that were subsequently noted DHA concentrations rose above pre-treatment levels in three patients (nos 1–3), and peaks occurred at 3 or 24 h after the first or second injection The mean concentration for all patients was however, not statistically different from the pre treatment mean In contrast, when the mean increment was calculated for all patients to compensate for rising basal concentrations with age, the mean increment at 3 h after the first injection and at 24 h after the second, was significantly higher than at any other time during the test (Table 2) There was also a positive correlation between individual basal levels and the sum of increments for the first or second 24 h after hGH (first 24 h, $r=0\ 71$, $p<0\ 05$, second 24 h, $r=0\ 73$, $p<0\ 05$)

Relationship of hGH increments to DHA increments during the test

The dose of hGH received (10 mg) by each patient led to varying serum concentrations of hGH It was necessary therefore, to calculate the increments of hGH achieved to determine whether the incremental rise in DHA obtained for some patients was related to individual increments of hGH

All peaks of hGH occurred 3 h after the first or second injection, but the levels reached

Table 3 *Increments of DHA and hGH after the first injection*

Pat no	Increment of DHA (nmol/l)	Increment of hGH (mμ/l)
1	+7 0	+370
2	+5 1	+129
3	+3 6	+242

varied from 31 to 460 mμ/l with a rapid decline to basal levels by 24–48 h after the first injection and by 24 h after the second The hGH increments after the first injection (sum 3–48 h values) ranged from +109 to +650 mμ/l and +52 to +655 mμ/l 3–24 h after the second

Patients (nos 1–3) showed increasing DHA increments coincident with rising increments of hGH after the first injection (Table 3) However, there was no statistical relationship between increments of DHA and hGH when the data from all the patients was analysed There was no correlation between age or bone age and increments of DHA, but the three patients in whom a rise in DHA was obtained were on average, older and their bone ages more advanced than those who failed to increase their DHA (mean age 16 75 years, mean bone age 12 0 years, compared to mean age 10 4 and mean bone age of 6 9 years)

DISCUSSION

The results of the present study suggest that the acute administration of hGH can elicit an increase in the concentration of circulating DHA in some patients with growth retardation

The DHA increments rose in three children and coincided with an incremental rise of hGH after the first injection However, those children whose DHA did not rise, had similar increments of hGH It is concluded therefore, that the absence of a DHA response is not related to an inadequate level of hGH reached during the test The only major clinical difference between responders and non responders

Acta Pædiatr Scand 69, 293–297, 1980

TREATMENT OF GIRLS WITH EXCESSIVE HEIGHT PREDICTION

Follow up of Forty Girls Treated with Intramuscular Estradiol and Progesterone

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ABSTRACT Andersen, H , Brock Jacobsen, B , Kastrup, K W , Krabbe, S , Peitersen, B , Petersen, K E , Thamdrup, E and Wichmann, R (Endocrine Clinic, Children s Hospital Fuglebakken University of Copenhagen) Treatment of girls with excessive height prediction Follow up of 40 girls treated with intramuscular estradiol and progesterone Acta Pædiatr Scand, 69 293, 1980.—In a follow up study of 40 tall girls treated with intramuscular estradiol and progesterone, the final height, bone age maturation, side effects and acceptance of treatment were evaluated The mean duration of treatment was 18 months During treatment, mean height increase was 6 5 cm (height velocity 3 7 cm/year), which is nearly 50% reduction of normal growth rate The mean increase in bone age was 2 7 years (bone age velocity 1.8 years/year), which approximates twice the normal maturation rate The mean reduction in final height was 5 0 cm as evaluated by the method of Bayley Pinneau (BP) 2 9 cm by the method of Tanner et al (TW) and 3 0 cm by the method of Roche et al (RWT) The reduction was greatest when treatment was started before menarche according to all three prediction methods When treatment was started after menarche the calculated height reduction was greatest according to the BP method There was good agreement between the three prediction methods in girls with a bone age below 12 years before treatment In girls with a bone age above 12 years the height reduction by the BP method was much greater than when measured by the other methods Side effects evaluated at follow up were minimal and first menstruation occurred within 3 months (mean) after cessation of treatment. The number of pregnancies was estimated to be normal for age All but three accepted the treatment It is concluded that this type of treatment must be restricted to girls with severe psychological problems due to excessive height prognosis and selection for treatment must be based on an individualized evaluation

KEY WORDS Height prediction, tall girls estrogen treatment

The problem in relation to estrogen treatment of tall girls was thoroughly discussed recently at a conference on estrogen treatment of the young (5)

The present paper presents experience with estrogen treatment performed as intramuscular injections of a long acting estrogen ester and progesterone in 40 girls in whom adult height was anticipated to become unacceptably high by the girls and their parents Results of the treatment are evaluated by comparing three methods of predicting adult height The calculated reduction of adult height is compared with results in other series published Side effects are discussed as well as the acceptance of the treatment by the girls

MATERIAL AND METHODS

In the period 1965 to 1976 46 girls with a *predicted adult* height above 175 cm (the majority above 180 cm) according to the methods of Bayley & Pinneau (2) and Tanner et al (8) *were treated* for more than 6 months The selection for treatment was based on the height prediction and the psychological impact on the girls and their parents *None* of the girls had clinical signs of endocrine disorders Six girls had to be excluded 2 could not join the follow up and in 4 the *results* of the bone age determination before treatment were not available 40 girls were seen at the follow up

Bone age determinations were carried out according to

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Submitted Aug 7 1979
Accepted Nov 19 1979

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Table 3 *Height reduction* (cm) *in 40 girls treated before or after menarche*

	Method		
	Bayley & Pinneau	Tanner et al	Roche et al
Before menarche			
Mean	3 9	3 8[a]	3 4
S D	±5 2	±3 0	±2 9
Range	(−2 0 to 13 8)	(−3 1 to 11 5)	(−0 6 to 12 3)
n	31	29	20
After menarche			
Mean	4 3	1 2[a]	1 8
S D	±4 2	±3 1	±2 7
Range	(−0 1 to 11 1)	(−4 0 to 5 9)	(−1 5 to 5 8)
n	9	9	9

[a] Statistically significant different ($p<0\ 02$)

Bone age at start of treatment in relation to the calculated reduction of final height is seen in Table 4 In girls with a bone age below 12 years there is good agreement between the three prediction methods With a bone age above 12 years the calculated height reduction is much greater according to the BP method than to the other methods In girls with a bone age less than 12 years at start of treatment the calculated height reduction is more marked according to the TW method than in girls with a more advanced bone age

Acceptance of the treatment At follow up the girls were questioned as to their opinion of the treatment All but three expressed satisfaction Two of the 3 dissatisfied girls found that the treatment had been very troublesome and their reason for completing it was to please their parents The third girl was now opposed to treatment and would have preferred a natural pubertal development

Side effects

During treatment In 5 girls the dose of estradiol progesterone, or both was reduced because of irregular and heavy bleedings, one of the girls also complained of nausea and dizziness

Minor side effects which did not involve alteration of the treatment were seen in 14 girls striae, disturbing weight gain, some irregular bleedings, transient nausea, headache and psychological problems

At the follow up examinations None of the girls had developed thromboembolic incidents or diabetes Thirty one girls menstruated after 1–2 months and 7 girls after 3–6 months One girl had amenorrhoea for 12 months but is now menstruating regularly, and one girl still has amenorrhoea 22 months after cessation of treatment With these exceptions none of the patients complained of gynaecological problems

None of the patients complained of sterility 5 had been pregnant (four births and one abortion) Nineteen used contraceptive pills

DISCUSSION

Papers published by Bierich, Crawford, Prader & Zachmann (5) and Wettenhall et al (9) have reviewed the literature on the subject and the results of series published earlier

In earlier series the patients have been treated orally with diethylstilbestrol, ethinyl estradiol or conjugated estrogens In the

Table 4 *Height reduction* (cm) *in relation to bone age (BA) at the beginning of treatment*

	Method		
BA (year)	Bayley & Pinneau	Tanner et al	Roche et al
<12			
Mean	4 5	5 0[a]	4 4
S D	±3.5	±3 0	±3 9
Range	(−1 to 13 8)	(−2 to 11 5)	(−0 6 to 12 3)
n	13	13	7
12–13			
Mean	6 3	3 6	1 7
S D	±3 8	±2 1	±2 0
Range	(−2 0 to 11 1)	(−0 1 to 8 1)	(−0 4 to 5 8)
n	12	12	10
>13			
Mean	4 4	0 7[a]	3 3
S D	±3.5	±3 4	±2 6
Range	(0 to 10 9)	(−4 0 to 4 8)	(−1 5 to 6 9)
n	15	14	9

[a] Significant difference ($p<0\ 01$)

Table 1 *Chronological age, bone age and height in 40 girls before and after treatment and at follow up*

	Chrono-locical age (year)	Bone age (year)	Height (cm)
Beginning of treatment			
Mean	12 6	11 8	171 1
S D	±1 4	±1 0	±8 1
Range	(10 2 to 16 3)	(8 0 to 13 6)	(145 to 184)
After treatment			
Mean	14 1	14 5	177 6
S D	±1 1	±0 6	±4 7
Range	(11 9 to 17 0)	(13 to 15 5)	(164 to 188)
At follow up			
Mean	18 5	>15*	179 8
S D	±3 4		±4 6
Range	(13 3 to 27 9)	(14 to 18)	(166 5 to 191)

* Three girls had a bone age of 15 years one girl had a bone age of 14 years

Greulich & Pyle (4) Radiographs of the left hand evaluated by one observer (E T) *Height predictions* were performed according to Bayley & Pinneau (BP) (2) Tanner et al (TW) (8) and Roche et al (RWT) (6)

The mean values of chronological age bone age and height at start and withdrawal of treatment and at the follow up are given in Table 1 At the start of treatment the pubertal stages according to Tanner (7) were in 4 girls stage 1 in 26 girls stage 2 and in 10 girls stages 3–4 Nine girls had had menarche before starting treatment

Treatment The girls received weekly intramuscular injection with estradiol valerate (Progynon® Depot) 10 mg for 3 weeks the third injection supplemented with oxyprogesterone caproate (Proluton® Depot) 125 mg The next series of injections was given after an interval of 2 weeks thus giving a total amount of estradiol valerate of 30 mg per month As a rule the injections were given in the clinic A few were treated by their general practitioner for geographical reasons but seen regularly at the clinic

Follow up examinations The girls were interviewed and had a physical examination at the clinic Thirty six girls had a chronological age above 18 years or a bone age above 15½ years in 3 the bone age was 15 years and in one 14 years These latter 4 patients had increased only 0–1 cm in height during 5–16 months after follow up

RESULTS

The mean values of chronological age, bone age and height at the start and withdrawal of treatment and at the follow up are seen in Table 1 The average duration of treatm was 18±7 6 months (mean±S D)

During treatment the height increa 6 5 cm (mean value) with a height velocit 3 7 cm (cm/year), which is a reductior nearly 50% of the normal growth rate for g of a comparable age (1) The mean advar ment of bone age was 2 7 years (Table 1) T equals a bone age velocity of 1 8±0 8 yea year or approximately twice the normal sc tal maturation rate

The average height reduction was evalua by comparing results of the three predict methods The reduction was calculated as difference between the predicted final hei and height at follow up for each girl Me values for reduction in final height using three prediction methods are shown Table 2 It appears that good agreement exi between prediction values obtained by methods of TW and RWT, whereas the method predicts higher values and, therefo an apparently greater reduction in final heig This was also found by Colle et al (3)

The influence of menarche before or af start of treatment is seen in Table 3 In pr menarchial girls the reduction in final heig was greater than in girls whose treatme started after menarche, and there is goo agreement between the three predicti methods In the post menarchial girls the ca culated height reduction is greater accordi to the BP method than to the two oth methods

Table 2 *Height reduction (cm) in 40 girls*

	Method		
	Bayley & Pinneau	Tanner et al	Roche et al
Mean	5 0	2 9	3 0
S D	±3 7	+3 4	±2 9
Range	(−2 to 13 8)	(−3 1 to 11 5)	(−1 5 to 12 3)
n	40	38	26

$p<0.02$ (Bayley & Pinneau vs Tanner et al)

$p<0.025$ (Bayley & Pinneau vs Roche et al)

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Submitted July 25, 1979
Accepted Oct 15, 1979

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present series estradiol valerate was chosen as the most physiologically suitable form of treatment, since estradiol valerate is metabolized to 17 beta-estradiol ('natural estrogen') It is well-known that daily intake of tablets is often neglected, whereas the injections ensure that the selected dose is given We therefore used estradiol valerate in oil as a long acting medication The weekly contacts with the patients offered frequent opportunities to discuss possible side effects and psychological problems Acceptance of the more troublesome intramuscular injections might indicate that the girls were highly motivated for treatment

The reduction in the final height achieved in this series is not very different from other series, in which the prediction of the final height has been estimated according to the BP method In the series by Zachmann et al (10) the mean reduction of height was 4 6 cm according to the TW prediction, whereas in the present series it was only 2 9 cm

The effect of the treatment might be dose-related, but it is difficult to compare different preparations which are metabolized in different ways However, based on experiences from gynaecological practice (Lebech, personal communication) the doses stated by Prader & Zachmann (5) could be considered in the following way ethinyl estradiol 0 3–0 5 mg per day as a high dose, conjugated estrogens 5–15 mg per day as a medium to high dose, and estradiol valerate 30–60 mg per month as a medium dose The patients treated by Zachmann et al (10) received 0 3 mg ethinyl estradiol per day (9 0 mg per month) The patients in the present series received 30 mg estradiol valerate per month The Zurich group (5) has recently started a pilot study in which an intramuscular injection of 40 mg estradiol valerate is followed 2 weeks later by an intramuscular injection of estradiol benzoate 10 to 40 mg and 17 hydroxyprogesterone caproate 250 mg

The greatest reduction in final height is obtained in the youngest girls and in girls with a low bone age In this series the mean height reduction was 5 cm according to the TW prediction in girls with a bone age below 12 years but only 0 7 cm in girls with a bone age above 13 years In girls who had menstruated before the start of treatment the mean reduction was only 1 2 cm

Only minor side effects of the treatment were seen All but two girls had spontaneous menstruation less than 6 months after cessation of the treatment The number of pregnancies at the time of follow up is not remarkably low, considering the young age of the girls and the fact that 19 had used contraceptive pills Development of endometrial carcinoma has been described in younger women, using estrogens as a sequence medication for contraception, but has not been published in tall girls treated with estrogens This risk cannot be entirely excluded before larger series of treated girls have been followed for a longer period

For psychological reasons it is not acceptable to start estrogen treatment before signs of pubertal development have appeared—we recommend pubertal stage 2 The prediction methods of TW or RWT seem to be the most reliable for predicting height in tall girls as also shown by Zachmann et al (11) The treatment must be restricted to girls with severe psychological problems concerning their height and/or with a height prediction at least above 180 cm and only if the treatment can be started at an early stage of pubertal development preferably at a bone age below 12 years We are in agreement with the consensus of the discussion during the conference on estrogen treatment in the young (5), that individualization is of utmost importance and that no universally applicable guide lines are available

ACKNOWLEDGEMENTS

Progynon® Depot and Proluton® Depot were placed at our disposal by Schering Kemi A/S Chief physician P E Lebech M D Frederiksberg Hospital kindly helped with the evaluation of the different estrogen doses

Acta Paediatr Scand 69 299–304, 1980

SERUM PROLACTIN LEVELS IN RENAL INSUFFICIENCY IN CHILDREN

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ABSTRACT Ijaiya, K, Roth, B, and Schwenk, A. (The University Children's Hospital, University of Cologne, Cologne, Federal Republic of Germany) Serum porlactin levels in renal insufficiency in children Acta Pa ... centrations in forty-eight children with ... with those in thirty four healthy contr ... abnormal prolactin response to intrave... children with chronic renal failure on maintenance intermittent haemodialysis No significant change in plasma prolactin concentrations and osmolality was observed before and after haemodialysis, despite a fall in plasma creatinine concentrations. The elevated prolactin levels fell to normal in three patients after successful renal transplantation. It is suggested that the kidney has an important role to play in prolactin metabolism.

KEY WORDS Prolactin, thyrotropin releasing hormone, renal disease, uraemia, haemodialysis

In addition to its well recognized regulation of mammary gland function, prolactin (PRL) also appears to exert a regulatory influence on several other extramammary target organs Evidence in lower animals that PRL regulates certain aspects of salt and water metabolism (17, 22, 33) has led clinical investigators to look for similar actions in man Elevated PRL levels have been reported in patients with chronic renal failure (8–10, 23, 28), and about 20–32% of adult patients exhibit hyperprolactinaemia (8, 9) Nevertheless, the importance of these observations is not clear, as other peptide hormones do not show increased levels in this condition, these high levels of prolactin cannot be simply due to poor glomerular filtration or inability of the kidney to degrade these hormones (23) Other authors have reported that PRL is not an important osmoregulatory hormone in man (1, 2, 3, 28) The hyperprolactinaemia of renal failure, may be attributed in part, to altered renal metabolism and a deranged hypothalamic pituitary control mechanism in chronic uraemia (9, 23)

In the present study we have determined the prevalence of elevated PRL levels in acute and chronic renal disease Moreover, the response of PRL to intravenous administration of thyrotropin-releasing hormone (TRH) in some of the patients has also been evaluated

PATIENTS AND METHODS

Plasma PRL samples were determined in two groups of children with renal disease 1) Thirty patients (15 M, 15 F), aged 0 4–13 years with normal renal function, but renal disease of the following pathologies acute glomerulonephritis ($n=8$), acute pyelonephritis ($n=15$), recurrent urinary tract infection ($n=7$) None of the children received prednisolone, phenothiazines or antihypertensive drugs, they were all treated with antibiotics 2) Eighteen patients (9 M, 9 F) aged 7 4–16 9 years with impaired renal function and on maintenance intermittent haemodialysis the period of haemodialysis before the present investigation ranged from 0 5 to 9 years Haemodialysis was usually performed 5 h three times a week The causes of the renal failure were chronic pyelonephritis ($n=8$), chronic glomerulonephritis ($n=3$) renal hypoplasia ($n=2$) and in 5 patients respectively Alport's syndrome, oxalo-

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In memory of the late Prof Dr H J Andersen, Copenhagen

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Table 2 *Plasma prolactin, urea, creatinine, sodium, potassium and calcium levels as well as plasma osmolality in patients with chronic renal failure undergoing haemodialysis*

Given are the arithmetic mean values ($\bar{x}$) and standard deviations (S.D.)

	PRL (ng/ml)	Urea (mmol/l)	Creatinine (µmol/l)	Na^+ (nmol/l)	K^+ (mmol/l)	Ca^{++} (mmol/l)	Osmolality (mOsmol)
Before haemodialysis							
$\bar{x}$	19.71	26.2	795	135.5	5.5	2.25	294.5
S.D.	14.44	5.3	178	3.2	0.8	0.1	18.0
After haemodialysis							
$\bar{x}$	22.60	8.0	320	135.0	3.5	2.65	292.8
S.D.	16.26	3.5	100	4.8	0.8	0.25	17.3

basal prolactin levels being 2.5 fold higher. Plasma creatinine and urea concentrations in control subjects, as well as in patients with acute renal failure and after successful renal transplantation were normal. No correlation was found between PRL and creatinine levels in these subjects. A normal PRL response after administration of TRH was observed in the controls, and in patients with acute renal disease and after successful renal transplantation. In contrast, the patients with chronic renal failure showed an abnormal response to TRH, the elevated basal PRL could not be stimulated after TRH injection (Fig. 1). The elevated PRL levels (50 and 40 ng/ml) decreased to 5.5 and 9.1 ng/ml in the two male patients after renal transplantation, and a normal PRL release after TRH injection was observed. The plasma PRL concentrations in the female patient before and after successful renal transplantation are shown in Fig. 2.

Results of PRL before and after haemodialysis in patients with chronic renal failure are given in Table 2. At the end of 2 1/2th h of dialysis PRL concentrations remained almost unchanged with respect to the basal values, and at the end of the 5th h of dialysis a slight, but statistically insignificant mean increase of 1.9 ng/ml was noted. Mean PRL levels in basal conditions before haemodialysis showed positive correlation to plasma creatinine concentration in these patients ($r = 0.582$, $p < 0.01$) but not to other parameters listed in Table 2. Before haemodialysis the plasma PRL level rose simultaneously with creatinine concentration in plasma, no such effect was observed after haemodialysis. No correlation existed between plasma PRL levels and the respective plasma osmolality. The osmotic pressure in plasma seems not to influence prolactin concentrations.

DISCUSSION

The present study demonstrates that elevated basal plasma prolactin levels are found in uraemic children with chronic renal failure on maintenance haemodialysis. This hyperprolactinaemia has been attributed to the chronic renal insufficiency or to the effect of such drugs like alpha methyldopa or reserpine (8, 9, 23, 28). By contrast, patients with acute renal disease without clinical or biochemical signs of chronic renal failure showed no elevation of plasma PRL concentrations. These findings suggest that renal disease, per se, also does not influence the secretion and metabolism of prolactin, but that a progressive rise in prolactin concentrations occurs as renal function deteriorates. The same observations were made by Chirito et al. (8) as well as Cowden and co-workers (9) in adult patients, and confirm the relative close correlation between the grade of renal insufficiency or levels of plasma creatinine and prolactin concentrations. The high plasma concentrations of PRL observed in uraemic patients persist even when the electrolyte disorders are corrected and the elevated creatinine concentrations decreased by haemodialysis. Sciarra et al. (28)

Table 1. *Plasma Prolactin concentrations in patients with renal disease with or without impaired renal function and in control subjects*

·ated basal PRL values are found in patients with acute renal disease

	Control subjects (n=34	Acute renal ddisease (n=30)	Chronic renal failure (n=18)	Renal trans plantation n=3
PRL (ng/ml)				
$\bar{x}$	8 89	7 62	19 71**	6 50
S D	2 77	2 01	14 44	2 24

sis, cystinosis, polycystic renal disease and juvenile nephronophthisis The children included both home and hospital dialyzed patients Two patients had been nephrectomized bilaterally, two children received such antihypertensive drugs like dihydralazine and alpha methyldopa, and two were taking furosemide

Blood samples for the determination of PRL were collected in the recumbent position, before dialysis and at 2 1/2th and 5th h of dialysis Plasma osmolality was meas ured before and after the dialysis

Basal PRL levels were determined in 3 children (2 M, 1 F), aged 14 15 and 17 years before and after renal transplantation The causes of the renal failure in the two boys were megacysticmegaureter syndrome and chronic glomerulonephritis, and in the 17 year old girl chronic pyelonephritis The two boys showed no signs of puberty, whereas in the girl breast and pubic hair development (Stage 3 according to Tanner) had occurred but she had not yet menstruated Thirty four healthy children (18 M 16 F), aged 0 2–15 3 years served as a control group No renal, liver or endocrine disease was detected None of these subjects was taking drugs known to affect PRL secretion Informed consent of the parents was obtained in all children

PRL responses to intravenous synthetic TRH (Rele fact,® Hoechst AG Frankfurt) were determined for 15 patients with unimpaired renal function (first group) for two patients after successful renal transplantation and for 20 control subjects The dosis generally given was 200 µg, except for infants under 1 year of age who received 100 µg All children were fasted overnight and bed rest was maintained during the test Blood samples were with drawn through an indwelling cannula inserted into an arm vein at 0, 15, 30, 45, 60 90 and 120 min after TRH injection The heparinized blood was immediately centri fuged at 4°C, and the plasma was stored at −20°C until assayed for PRL For evaluation of the results only plasma PRL concentrations after TRH administration were taken into consideration

Plasma PRL was determined by a double antibody radioimmunoassay using the RIA Kit supplied by Serono (Rome Italy) The detection limit of the assay was 1 5 ng/ml and all samples from any one patient were assayed simultaneously Intra assay coefficients of variation were 8 % and inter assay coefficients of variation were 11 4 % Plasma sodium, potassium and calcium were assayed by flame photometry, plasma urea and creatinine by colori metric methods Osmolality was measured with an osmo meter Statistical evaluations were done by Wilcoxon Man and Whitney s U Test for unpaired groups and using the linear regression and correlation analysis

RESULTS

Results of basal plasma PRL levels are shown in Table 1 Mean basal levels were identical in controls and in patients with acute renal disease A statistically significant difference was seen between these groups and the group of patients with chronic renal failure, the mean

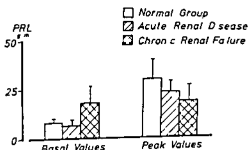


Fig 1 Plasma PRL concentrations before and after in travenous TRH administration Peak levels are shown after TRH injection The elevated basal plasma PRL con centrations could not be stimulated by TRH in chronic renal failure

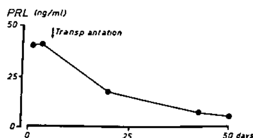


Fig 2 Basal plasma PRL concentrations in the 17 year old female patient before and after renal transplantation

Table 2 *Plasma prolactin urea, creatinine, sodium, potassium and calcium levels as well as plasma osmolality in patients with chronic renal failure undergoing haemodialysis*

Given are the arithmetic mean values (x) and standard deviations (S D)

	PRL (ng/ml)	Urea (mmol/l)	Creatinine (µmol/l)	Na* (nmol/l)	K* (mmol/l)	Ca** (mmol/l)	Osmolality (mOsmol)
Before haemodialysis							
x	19 71	26 2	795	135 5	5 5	2 25	294 5
S D	14 44	5 3	178	3 2	0 8	0 1	18 0
After haemodialysis							
x	22 60	8 0	320	135 0	3 5	2 65	292 8
S D	16 26	3 5	100	4 8	0 8	0 25	17 3

basal prolactin levels being 2 5 fold higher Plasma creatinine and urea concentrations in control subjects, as well as in patients with acute renal failure and after successful renal transplantation were normal No correlation was found between PRL and creatinine levels in these subjects A normal PRL response after administration of TRH was observed in the controls and in patients with acute renal disease and after successful renal transplanta tion In contrast, the patients with chronic renal failure showed an abnormal response to TRH the elevated basal PRL could not be stimulated after TRH injection (Fig 1) The elevated PRL levels (50 and 40 ng/ml) de creased to 5 5 and 9 1 ng/ml in the two male patients after renal transplantation and a normal PRL release after TRH injection was observed The plasma PRL concentrations in the female patient before and after successful renal transplantation are shown in Fig 2

Results of PRL before and after haemodia lysis in patients with chronic renal failure are given in Table 2 At the end of 2 1/2th h of dialysis PRL concentrations remained almost unchanged with respect to the basal values, and at the end of the 5th h of dialysis a slight, but statistically insignificant mean increase of 1 9 ng/ml was noted Mean PRL levels in basal conditions before haemodialysis showed positive correlation to plasma creatinine concentration in these patients (r=0 582, p<0 01) but not to other parameters listed in Table 2 Before haemodialysis the plasma PRL level rose simultaneously with creatinine concentration in plasma, no such effect was observed after haemodialysis No correlation existed between plasma PRL levels and the respective plasma osmolality The osmotic pressure in plasma seems not to influence prolactin concentrations

DISCUSSION

The present study demonstrates that elevated basal plasma prolactin levels are found in uraemic children with chronic renal failure on maintenance haemodialysis This hyperprolactinaemia has been attributed to the chronic renal insufficiency or to the effect of such drugs like alpha methyldopa or reserpine (8, 9, 23, 28) By contrast, patients with acute renal disease without clinical or biochemical signs of chronic renal failure showed no eleva tion of plasma PRL concentrations These findings suggest that renal disease, per se, also does not influence the secretion and metabolism of prolactin, but that a progressive rise in prolactin concentrations occurs as renal function deteriorates The same observations were made by Chirito et al (8) as well as Cowden and co-workers (9) in adult patients, and confirm the relative close correlation between the grade of renal insufficiency or levels of plasma creatinine and prolactin concentrations The high plasma concentrations of PRL observed in uraemic patients persist even when the electrolyte disorders are corrected and the elevated creatinine concentrations decreased by haemodialysis Sciarra et al (28)

also reported that changes in PRL did not correlate with the changes in the hydroelectrolyte balance and plasma aldosterone and renin concentration during haemodialysis Restoration of renal function after renal transplantation led to a reversion of prolactin concentrations towards normal (8, 9, 31)

These findings suggest that the hyperprolactinaemia of renal failure, is attributable, in part, to altered renal metabolism This mechanism may also explain the elevated concentrations of other hormones in uraemia, e g growth hormone (10, 18, 27) In man, there is evidence for the renal extraction of other peptides, e g insulin (14) and thyrotropin (TSH) (15) Donatsch & Richardson (13) have shown that in the rat, ovine prolactin gains access to the proximal tubular cells of the kidney by means of the glomerular filtrate The significance of the kidney for prolactin metabolism in man has been emphasized by Cowden and co workers (9) The kidney is supposed to be a clearance organ for prolactin, with a biologic half-life in serum of approximately fifteen minutes (30) A fall in the glomerular filtration rate with progressive renal failure could lead to a decline in plasma hormone clearance, and thereby resulting in hyperprolactinaemia

The exact mechanism is unclear, but a loss of renal degradation cannot be the only cause of elevated PRL concentrations in uraemic patients Other pituitary hormones such as growth hormone (GH), luteinizing hormone (LH) and follicle stimulating hormone (FSH) are elevated, whereas others like TSH are found normal or low in uraemic patients (10, 19, 23) Certain enzyme systems are inhibited by uraemia (16), and it is possible that enzyme systems which take part in PRL degradation may also be inhibited in uraemia Haemodialysis reduced azotaemia and normalised plasma calcium, potassium and sodium, but did not affect the plasma concentrations of PRL The failure of PRL to diminish post-dialysis would suggest that it is not being "driven" on an acute basis by solutes which are readily dialysable The high plasma PRL concentrations are also not due to changes in the hydroelectrolyte balance (28)

Furthermore, it seems that PRL secretion is disturbed as a result of an abnormal hypothalamic pituitary control mechanism in chronic uraemia TRH, a tripeptide (pyroglutamyl histidyl prolinamide) has been found to cause a marked release not only of TSH, but also of prolactin both in vitro and in vivo (4, 29) Since increased PRL secretion is due to a decrease in hypothalamic prolactin inhibiting factor (PIF) or to increase in prolactin releasing factor (PRF) (21), a dysfunction of hypothalamus may result in decreased PIF or increased PRF biosynthesis or secretion Czernichow et al (10) and Ijaiya (19) have noted an impaired response of PRL to TRH in children with chronic renal failure Chirito and co workers (8) have found that L dopa is unable to blunt PRL secretion by bromocriptine (24), suggesting a defect in the hypothalamic secretion of PIF in these patients The role of uraemic toxins or severe malnutrition in the abnormal hypothalamus pituitary secretion mechanism is not clear

The significance of elevated plasma PRL concentration in renal insufficiency in man is still not well understood Prolactin is a well established osmoregulatory hormone in fishes and amphibians (12, 20) This hormone is believed to affect the severity of the chronic progressive nephropathy seen in some species of rat (26) In man its role as an osmoregulator is still controversial Horrobin et al (17) demonstrated that PRL acts on the hydroelectrolyte balance, reducing renal excretion of water, sodium and potassium and increasing plasma sodium and osmolality Buckman and colleagues (5, 6) reported that serum PRL levels are related directly to plasma osmolality As to the renal sites of PRL action producing retention of salt and water direct effects on the proximal tubules distal tubules and collecting ducts have been suggested (14, 33) Other authors (1, 2, 3, 7, 11, 25 28, 32) have failed to confirm these relationships between prolactin and renal function The different re

ults reported by several authors in man may eflect species specificity or possibly contamination of administered PRL with vasopressin (2, 32)

The present results show that elevated PRL levels and an abnormal PRL response to TRH are found, like in adults, in children with chronic renal failure The positive correlation between PRL and creatinine and the return of elevated PRL to normal after successful renal transplantation suggest that the kidney plays an important role in the metabolism of prolactin

ACKNOWLEDGEMENTS

This study was supported by the Deutsche Forschungsgemeinschaft, Bonn We wish to thank Mrs Angelika Schmidt for the laboratory assistance

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also reported that changes in PRL did not correlate with the changes in the hydroelectrolyte balance and plasma aldosterone and renin concentration during haemodialysis Restoration of renal function after renal transplantation led to a reversion of prolactin concentrations towards normal (8, 9, 31)

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Acta Pædiatr Scand 69, 305–309, 1980

ALBRIGHT'S HEREDITARY OSTEODYSTROPHY

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From the First Paediatric Clinic and the Second Medical Clinic, University of Rome, Italy

ABSTRACT Boscherini, B., Coen, G., Bianchini, G., Gallucci, G., Ballanti, P, Pasquino, A. M, Piccolo, F., Manca Bitti, M. L. and Spadoni, G. L (First Paediatric Clinic and Second Medical Clinic, University of Rome, Italy). Albright's hereditary osteodystrophy. Acta Paediatr Scand, 69 305, 1980. The authors observed different clinical forms of Albright's hereditary osteodystrophy in 4 members of a family (two sisters, their mother and the maternal grandfather) The sisters were affected by pseudohypoparathyroidism type I, the older manifested the hypocalcemic variety, the younger the normocalcemic variety, the mother and the grandfather presented only with short stature and subcutaneous calcifications The variety of clinical and biochemical alterations observed in these 3 generations supports evidence that Albright's hereditary osteodystrophy has a broad spectrum and that distinctions between the various forms of pseudohypoparathyroidism should not be rigidly considered

KEY WORDS: Pseudohypoparathyroidism, Albright's hereditary osteodystrophy, PTH

The clinical syndrome characterized by short stature, obesity, mental retardation, brachymetacarpia, round facies, dental anomalies and subcutaneous calcifications is known as Albright's hereditary osteodystrophy (AHO) (1)

It can occur in subjects who do not show abnormalities in calcium and phosphate metabolism (pseudo-pseudohypoparathyroidism) or in patients in which a peripheral resistance to PTH can be demonstrated (pseudohypoparathyroidism)

The determination of plasma PTH, plasma and urine cyclic AMP and phosphate after PTH infusion and of serum calcium and phosphate concentrations enables one to distinguish the various forms of pseudohypoparathyroidism In type I, in which the defect lies in membrane receptors, exogenous PTH does not cause any increase in plasma or urine cAMP plasma PTH levels are high Calcium levels may either be normal or low, thus two varieties can be distinguished normocalcemic and hypocalcemic (3, 16), in type II, in which a defect inside the cell blocks the biologic effect of PTH (8), exogenous PTH fails to cause a normal increment in phosphaturia while cAMP response is normal

In some cases of pseudohypoparathyroidism the term hypohyperparathyroidism has been used since peripheral resistance to PTH is present only in the kidney, while bone sensitivity is conserved to the point of showing radiological signs of hyperparathyroidism (7).

We have observed different forms of clinical expression of AHO in 4 members of three generations of a family (two sisters, their mother and the maternal grandfather).

MATERIALS AND METHODS

Two sisters, the mother and the maternal grandfather were observed A complete study was possible only in the sisters Chemical analyses were done by standard methods

Plasma PTH was assayed in the older sister (case 1) by a radioimmunoassay method according to Fischer et al (9) normal values less than 40 ng/ml In the younger sister (case 2) and in the mother plasma PTH assay was carried out in our laboratory by a radioimmunoassay method, using antiserum against the C-terminal (35–84) fragment of PTH molecule and the bovine standard (Kit CEA Sorin IRE) normal values 1.38 ± 1.14 mU/ml

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Submitted July 27, 1979
Accepted Nov 10, 1979

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lasma cAMP pmol/ml		TRP %	
asal	After PTH max	Basal	After PTH max
8 4	9 4	99 20	94 0
8	30	92 10	72 0
2 9±5 1	187 7±126 6	94 93±3 1	63 16±23 70
	–	93 30±3 96	76 22±10 17

values) whereas renal Ca/Na clearance ratio decreased as in normal subjects and those affected by idiopathic hypoparathyroidism (15) thus showing a normal response of the tubule to PTH (Table 2) Biopsy of one of the subcutaneous nodule showed calcium deposits (Von Kossa ++++)

Patient 2 The second sister of patient 1 was delivered at term after an uneventful pregnancy Birth weight 3650 g Neonatal period normal The girl came to our clinic at age 1 year and 10 months Her weight (kg 15) and height (cm 90) were at the upper limits of the normal range (90th percentile) She had a round facies snub nose prominent forehead short and stubby hands and feet small hard nodules in the subcutaneous tissue of the trunk and limbs Psychomotor development was normal There were no signs of latent tetany EEG and ophthalmoscopic examination were normal X rays of the skeleton showed slight thickness of the frontal bone short stubby tubular bones of the hands small calcifications throughout the soft tissues of the limbs (Fig 3) A biopsy of the subcutaneous nodules showed calcium deposits

Serum calcium (2 2 5 mmol/l) serum phosphorus (1 4–1 6 mmol/l) and alkaline phosphatase (6 5–6 7 UBL) were normal PTH plasma level was 1 92 mU/ml

Like her sister the parathyroid extract infusion test showed a lack of response of urine and plasma cAMP

phosphatase (1 2 UBL) and PTH plasma levels (0 4 mU/ml) were all normal

The maternal grandfather showed short stature (145 cm) and a dark hard nodule (0 5 cm in diameter) on the lateral aspect of the left thigh A biopsy showed calcium deposits in the fascia lata

DISCUSSION

Our patients had different clinical and radiological expressions of AHO Short stature, mental retardation and short 4th and 5th metacarpal bones were missing in the youngest of the two sisters The mother and the grandfather manifested only short stature and subcutaneous calcifications

In both sisters administration of exogenous PTH did not cause any increase in urine or plasma cAMP concentrations, as it occurs in pseudohypoparathyroidism type I (16), in particular hypocalcemia and high plasma PTH concentration allow us to include case 1 in the hypocalcemic variety of pseudohypoparathyroidism, while the normal plasma PTH and calcium values indicate that case 2 is affected by the normocalcemic variety (3, 15)

Since it was impossible to test cAMP after infusion with PTH both in the mother and in the grandfather due to lack of consent, we cannot establish whether a peripheral resistance to PTH was present

The finding of normal calcium and plasma PTH concentrations in the mother supports the hypothesis that both she and the grandfather are affected by pseudopseudohypoparathyroidism or by the normocalcemic variety of pseudohypoparathyroidism type I, like the younger sister (case 2)

It must be stressed that even the biochemical anomalies, i e calcium and phosphorus metabolism, were different in our patients the

Table 2 *Urine Ca/Na clearance ratio in response to PTH administration in our patients two other subjects affected by pseudohypoparathyroidism and two affected by idiopathic hypoparathyroidism*

Patients	Urine Ca/Na clearance ratio Basal	30	60	120	180
Pseudohypoparathyroidism					
Case 1	1 46	0 25	0 16	0 26	0 08
Case 2	0 42	0 43	0 36	0 43	0 67
D R	0 64	0 68	–	1 31	0 85
B B	0 19	0 38	0 41	0 08	0 14
Idiopathic hypoparathyroidism					
F P	1 00	0 33	0 21	–	0 27
G G	1 95	0 97	–	0 31	0 79

Table 1 Laboratory data in 8 subjects affected by idiopathic hypoparathyroidism and 4 control

Patients	Calcemia (mmol/l)	Phosphoremia (mmol/l)	Plasma PTH	Urine cAMP nmol/ml Basal	Urine cAMP nmol/ml After PTH max
Case 1	1 7–2	2–2 7	253 ng/ml[a]	2 15	2 52
Case 2	2–2 5	1 4–1 6	1 92 mU/ml[b]	0 46	1 13
Id Hypoparathyroidism n°=8 (mean values±S D)	1 8±0 3	2 3±0 6	Undetectable	1 44±0 64	308+155
Controls n°=4 (mean values±S D)	2 4±0 02	1 6±0 2		4 02±0 69	286 7±148 8

[a] Normal values<40 ng/ml [b] Normal values 1 38±1 14 mU/ml

Urine and plasma cAMP response to PTH infusion was tested in the two sisters After overnight fasting the patients received 100 200 ml of water at the beginning of the study and 50–100 ml hourly thereafter to ensure adequate urine output After two 60 min basal urine collections and baseline blood samples 8 units USP/kg of Paratormone Lilly 100/ml were administered intravenously over 2 min Urine and blood samples were again collected at 30 60 120 and 180 min after administration of the parathyroid extract Blood for cAMP determination was drawn before and at 10 20 30 and 50 min after parathyroid extract infusion Blood samples (5 ml) were collected in cooled tubes containing 50 μl EDTA 0 5 M to inhibit endogenous phosphodiesterase and after separation the plasma was immediately frozen to −70°C Urine and plasma cAMP was assayed by a competitive protein binding method (10)

Renal sensitivity to PTH was also tested determining phosphate urinary excretion as TRP% and renal Ca/Na clearance ratio according to Moses et al (15)

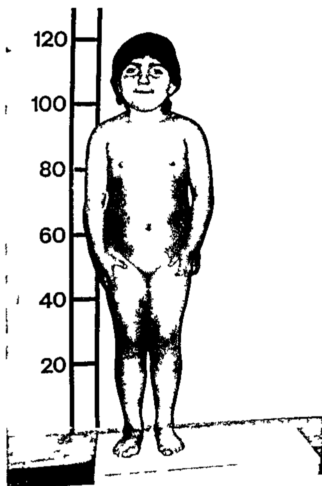


Fig 1 Patient 1

CASE REPORTS

Patient 1 (Fig 1) The first child of unrelated parents was delivered at term following an uneventful pregnancy Birth weight 3 350 g Neonatal period was normal The second child also a female died at the 11 months of age manifesting macrosomy and cardiomegaly The third child is our patient 2

The girl was admitted to our ward at age 10 years for short stature and mild mental retardation Her height was 120 cm (10th percentile) weight was 34 5 kg (90th percentile) she had a round facies snub nose an overall stubby aspect especially noticeable in the 3rd 4th and 5th fingers

tion There were no signs of latent tetany EEG showed mild bioelectric immaturity Ophthalmoscopic examination was normal X rays of the skeleton showed macrocephaly with thick parietal bones dysmorphic hand bones with small and deformed carpal nuclei short and stubby

generalized osteoporosis Small streaky calcifications were present in the soft tissues especially of the left thigh and leg and of the right foot

Even in the same patient symptoms and metabolic alterations may change with time for example the shortness of the 4th and 5th metacarpal bones may be absent in the first years of life (14) and be present only later, after the precocious fusion of the metaphysis has prevented these bones from growing as much as the others; patients have also been described who from normocalcemic have become hypocalcemic (2, 3, 4, 14, 16) and others whose plasma PTH concentrations have increased during the years, which has given rise to the hypothesis of an "early latent stage" of the disease (16)

The observations made by us and those of other authors indicate that the spectrum of AHO is very wide and that distinctions between various forms of pseudohypoparathyroidism should therefore not be considered in a rigid and definitive way

Lastly, the phenomenon that AHO can manifest itself in different ways in members of the same family belonging to three generations, confirms, on one hand, that the syndrome is hereditary, but on the other hand suggests that the modes of inheritance are in reality more complex than presently thought.

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Submitted June 27, 1979
Accepted Oct 11, 1979

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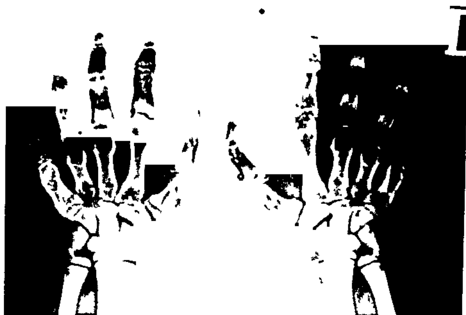


Fig 2 X ray film of the hand of patient 1 Short 3rd 4th 5th meta carpal bones are evident

mother and the younger sister were normocalcemic and had normal plasma PTH levels, while the older sister was hypocalcemic and had high plasma PTH levels After infusion with PTH, cAMP response was in both sisters blunted, in the older (case 1) Ca/Na clearance ratio decreased normally, while in the younger (case 2) no change was observed

Paradoxically in patient 2 normal phosphaturic response was observed this equivocal result is occasionally found in subjects affected by pseudohypoparathyroidism (5), especially of the normocalcemic variety (16)

The variation of expression of AHO in subjects belonging to the same family indicates that many biochemical alterations in calcium and phosphorus metabolism in patients with this disease are possible Similar observations are reported in the literature patients belonging to the same family with different clinical symptoms and metabolic alterations have been described by Mann et al (12), and Chase et al (5) studied two brothers affected by pseudohypoparathyroidism whose mother was affected by pseudo pseudohypoparathyroidism



Fig 3 X ray film of the hand of patient 2 Short and stubby tubular bones and subcutaneous calcifica

Acta Pædiatr Scand 69 311–313, 1980

ENHANCED RENAL TUBULAR CALCIUM REABSORPTION INDEPENDENT OF PARATHORMONE ACTIVITY, IN CHILDREN ON LONG TERM ANTICONVULSANT THERAPY

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ABSTRACT Aladjem, M , Shohat, M , Orda S , and Boichis, H (Pediatric Renal Unit, Chaim Sheba Medical Center, Tel Hashomer, and the Sackler School of Medicine, Tel Aviv University Israel) Enhanced renal tubular calcium reabsorption, independent of parathormone activity, in children on long term anticonvulsant therapy Acta Paediatr Scand, 69 311 1980.—A significant reduction in the urinary calcium-creatinine ratio, was found in thirty four ambulatory epileptic children on long term anticonvulsant therapy, studied during wintertime No significant difference in the urinary excretion of cyclic AMP, phosphate or amino-acids was observed when the study group was compared to an age-matched control group Serum calcium and phosphate values were normal, but a significant increment in alkaline phosphatase was noted Since there was no evidence of hyperactivity of the parathormone, it is suggested that the enhanced renal tubular calcium reabsorption in children on long term anticonvulsant therapy is a nonparathormone mediated phenomenon possibly compensating for reduced calcium absorption in the gastrointestinal tract

KEY WORDS Calcium anticonvulsants cyclic AMP

Elevated serum values of alkaline phosphatase (A P) in epileptic adults on long term anticonvulsant therapy were initially reported by Wright (20) while overt manifestations of rickets among such patients were described originally by Schmid (16) and later confirmed by Kruse (12) Several large surveys of this patient population showed many of the parameters of rickets decreased serum calcium and elevated A P (3 8 10 14 15, 19), hypophosphatemia (3 8, 19) hyperaminoaciduria (14) reduced urinary calcium excretion (14) and radiological evidence of rickets (3, 14) A reduction in the urinary excretion of calcium associated with low serum concentrations of calcium and phosphate was reported by Lifshitz (14) Plasma levels of parathormone (P T H) in patients on anticonvulsant therapy with biochemical evidence of rickets, were found to be within normal limits (19) Renal excretion of cyclic AMP was never investigated previously in such cases to our knowledge We report a survey performed during wintertime on 34 epileptic pediatric patients on anticonvulsant therapy in Israel, in whom PTH activity was estimated by the urinary excretion of cyclic AMP, and in whom other parameters of calcium and phosphate metabolism were measured concomitantly

MATERIALS AND METHODS

Thirty four ambulatory epileptic children 19 boys and 15 girls who had been on anticonvulsant therapy [illegible] years (mean 8 6 y ±3 9 y) All the patients underwent a full physical examination A first morning urine sample was obtained and a blood sample was drawn Wrist X rays were performed on all patients Serum calcium was determined by Corinth Calcium Clinton Lab Syst (1) creatinine was determined by the Jaffe reaction phos

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This study constitutes part of the requirements for an M D Thesis at the Sackler School of Medicine Tel Aviv University

ENHANCED RENAL TUBULAR CALCIUM REABSORPTION INDEPENDENT OF PARATHORMONE ACTIVITY, IN CHILDREN ON LONG TERM ANTICONVULSANT THERAPY

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ABSTRACT Aladjem M, Shohat, M, Orda, S, and Boichis, H (Pediatric Renal Unit, Chaim Sheba Medical Center, Tel Hashomer, and the Sackler School of Medicine, Tel-Aviv University, Israel) *Enhanced renal tubular calcium reabsorption, independent of para thormone activity, in children on long term anticonvulsant therapy.* Acta Paediatr Scand. 69 311, 1980.—A significant reduction in the urinary calcium-creatinine ratio, was found in thirty four ambulatory epileptic children on long term anticonvulsant therapy, studied during wintertime No significant difference in the urinary excretion of cyclic AMP, phosphate or amino-acids was observed when the study group was compared to an age-matched control group Serum calcium and phosphate values were normal, but a *significant increment* in alkaline phosphatase was noted Since there was no evidence of hyperactivity of the para thormone, it is suggested that the enhanced renal tubular calcium reabsorption in children on long term anticonvulsant therapy is a nonparathormone mediated phenomenon, possibly compensating for reduced calcium absorption in the gastrointestinal tract

KEY WORDS Calcium, anticonvulsants, cyclic AMP

Elevated serum values of alkaline phosphatase (A P) in epileptic adults on long term anticon vulsant therapy were initially reported by Wright (20) while overt manifestations of rickets among such patients were described originally by Schmid (16) and later confirmed by Kruse (12) Several large surveys of this patient population showed many of the para meters of rickets decreased serum calcium and elevated A P (3, 8 10, 14, 15, 19), hypo phosphatemia (3 8 19), hyperaminoaciduria (14) reduced urinary calcium excretion (14), and radiological evidence of rickets (3, 14) A reduction in the urinary excretion of calcium associated with low serum concentrations of calcium and phosphate was reported by Lif shitz (14) Plasma levels of parathormone (P T H) in patients on anticonvulsant therapy, with biochemical evidence of rickets, were found to be within normal limits (19) Renal excretion of cyclic AMP was never in vestigated previously in such cases to our knowledge We report a survey performed during wintertime on 34 epileptic pediatric patients on anticonvulsant therapy in Israel, in whom PTH activity was estimated by the urinary excretion of cyclic AMP, and in whom other parameters of calcium and phosphate metabolism were measured concomitantly

MATERIALS AND METHODS

Thirty four ambulatory epileptic children 19 boys and 15 girls who had been on anticonvulsant therapy for the duration of 2 to 7 years(mean 4 years) were referred to the pediatric renal unit Treatment cons sted of [illegible]

[illegible] ned by Corinth Calcium Clinton Lab Syst (1) creatinine was determined by the Jaffe reaction phos-

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cium adequately compensates for the reduced gastrointestinal absorption of this ion in our patients The lack of overt biochemical or radiological signs of rickets is probably due to an enhanced exposure to sun in this country

It is suggested that the earliest manifestation of reduced gastrointestinal absorption of calcium in children on anticonvulsant therapy is a PTH independent enhancement of tubular reabsorption of this ion This compensatory mechanism initially prevents the development of a negative calcium balance It seems likely that factors such as reduced exposure to sun light and/or reduced or inadequate diet may offset this delicate balance resulting in the subsequant development of rickets

ACKNOWLEDGEMENT

Thanks are due to Dr Aliza Eshkol and the staff of the Endocrine Institute at our Medical Center for kindly per forming the Cyclic AMP measurements on our subjects

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Submitted July 13, 1979
Accepted Oct 5, 1979

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Table 1. *The biochemical results of 34 epileptic patients and 35 controls*

	Epileptic group (Mean±S D)	Control group (Mean±S D)
Serum calcium, mmol/l	2 6±0 2	2 6±0 1
Serum phosphate, mmol/l	1 3±0 3	1 3±0 2
TRP,	92 0±3 7	92 6±5 0
Cyclic AMP, pmol/mg Cr	42 8±18 2	40 2±13 2
Urinary Ca/Cr ratio	0 072±0 045[a]	0 118±0 075[a]
A P , mu/ml (international units)	225±68[b]	181±57[b]

[a] Significant to $p<0\ 01$
[b] Significant to $p<0\ 05$

phate by the Fiske & Subarow method (5), aminoacids were determined semiquantitatively by paper chromatography, and cyclic AMP by the method of Gilman (6) Creatinine clearance was calculated using Schwartz s formula (17) Thirty five healthy children, 18 boys and 17 girls, 3 to 17 years of age (mean 9 4 y ±4 3 y), studied during the same months as their epileptic counterparts, served as controls The controls underwent identical physical and laboratory examination but did not have X rays of their wrists The student *t* test was employed in order to determine statistical significance

RESULTS

No significant differences were observed between the study group and the control group when height, weight, serum calcium, serum phosphate, tubular reabsorption of phosphate (TRP) and urinary excretion of cyclic AMP were studied (see Table 1) Creatinine clearance was normal in all subjects and similar in both the controls and the study group

Urinary aminoacid excretion was normal in both groups However, the urinary Calcium/creatinine ratio of the study group, 0 072±0 045, was markedly lower than that of the control group 0 118±0 075 ($p<0\ 05$) (see Table 1) No radiological evidence of rickets was demonstrated in either group No correlation was observed between urinary cyclic AMP, serum A P and urinary Ca/Cr ratio, and the Ca/Cr ratio did not correlate significantly with the duration of anticonvulsant therapy

DISCUSSION

A higher incidence of rickets among epileptic patients on anticonvulsants has been reported previously by several investigators (3, 8, 10 12, 14, 15, 16, 19) Rapid disappearance and low serum 25-hydroxycholecalciferol levels were observed by Hahn (8) An increased catabolism of Vitamin D and 25-hydroxycholecalciferol secondary to induction of liver microsomal enzymes by the anticonvulsant drugs has been suggested by Dent as a possible explanation for these phenomena (4) Recently however, normal or moderately elevated 1–25 dihydroxycholecalciferol values were demonstrated in patients on anticonvulsant therapy (11) Such findings are compatible with a reduced 1–25-dihydroxycholecalciferol effect on intestinal calcium absorption, and indeed in several studies the addition of anticonvulsant drugs reduced calcium absorption from the gut in the presence of vitamin D (2, 9, 12) None of the patients examined by us had clinical or radiological signs of rickets, in the face of a significant reduction of the urinary Ca/Cr ratio Reduced urinary Ca/Cr ratios were previously reported by Lifshitz (14), but only in patients with significantly lower levels of serum calcium The finding of a reduction in urinary Ca/Cr ratio in the presence of normocalcemia suggests that an increased tubular reabsorption of calcium may have occurred in patients on anticonvulsant therapy

This state of affairs could conceivably arise from an increased activity of the PTH, a well known moderator of renal tubular calcium reabsorption, however, both the normal PTH levels reported by others in similar cases (19), and the normal urinary cyclic AMP excretion observed in this study, mediate against such a hypothesis

Increased AP values, such as were described in our study, in the absence of PTH hyperactivity have been attributed to general induction of liver enzymes by the anticonvulsant drugs (18, 15)

It seems that the renal conservation of cal-

Acta Pædiatr Scand 69. 315–320, 1980

CYTOTOXIC TREATMENT IN CHILDREN WITH IDIOPATHIC NEPHROTIC SYNDROME

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ABSTRACT Herin, P and Eriksson, M (Department of Paediatrics, Karolinska Institutet, St Goran's Children's Hospital, Stockholm, Sweden). Cytotoxic treatment in children with idiopathic nephrotic syndrome. Acta Paediatr Scand, 69 315, 1980.—The purpose of this retrospective study was to investigate the therapeutic value of steroids and [illegible]

the latter two groups therapy was less successful than in the MCNS-group. This study conclusively demonstrates that cytotoxic therapy is of value in prolonging the duration of the remission and increasing the responsiveness to steroids in children with MCNS.

KEY WORDS Nephrotic syndrome, cytotoxic drugs

Corticosteroids (steroids) are commonly used to induce remissions in the idiopathic nephrotic syndrome in children (13, 21) The renal morphology is of major importance in determining the clinical course and the response to therapy In the majority of patients the immediate response is good but the long-term effect is uncertain (1, 11, 24) Some patients, however, do not respond to treatment and/or may develop serious side effects following repeated or prolonged therapy In the desire to improve the outcome other drugs, in particular cytotoxic compounds (2, 3, 5, 7, 14 17), have been tried Considerable experience has now been gained in the treatment of various types of glomerular disease with cytotoxic drugs This regards especially patients who respond to steroids but have frequent relapses or are refractory to steroids alone

In the present report, the therapeutic response to steroids and cytotoxic drugs has been evaluated by examining renal biopsy specimens in children who have developed the idiopathic nephrotic syndrome during the past ten years. The purpose has been to determine whether cytotoxic drugs prolong the duration of the remission and improve the steroid response

MATERIALS AND METHODS

Thirty eight children who fulfilled the criteria for the idiopathic nephrotic syndrome and developed this condition during the period 1968–1977 were included in the study The diagnosis was based on the presence of the classical clinical and laboratory features of severe proteinuria, hypoalbuminemia (25 g/l) and/or edema

Hypertension was defined as a diastolic pressure greater than 2 SD above normal values for age (19) Renal insufficiency was defined as a serum urea level above 8 g/l which was still present after the acute or relapsing period of the disease

[illegible]

fluorescent microscopy Five different antisera were used in the immunofluorescent examination (4)

The patients were divided into three categories based on the histopathologic findings The first two were classified in accordance with previous definitions (10)

1 Minimal change nephrotic syndrome (MCNS) (n=

Dedicated to prof Rolf Zetterstrom on the occasion of his 60th anniversary

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Corticosteroids (steroids) are commonly used to induce remissions in the idiopathic nephrotic syndrome in children (13 21) The renal morphology is of major importance in determining the clinical course and the response to therapy In the majority of patients the immediate response is good but the long term effect is uncertain (1 11 24) Some patients, however, do not respond to treatment and/or may develop serious side effects following repeated or prolonged therapy In the desire to improve the outcome other drugs, in particular cytotoxic compounds (2, 3, 5, 7 14 17) have been tried Considerable experience has now been gained in the treatment of various types of glomerular disease with cytotoxic drugs This regards especially patients who respond to steroids but have frequent relapses or are refractory to steroids alone

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At least one percutaneous renal biopsy was obtained

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Table 1 *Relative number of patients with persistent clinical complications and with positive immunofluorescent reactions (positive IFL) in the three morphologic groups of nephrotic children*

	MCNS (n=34)	FSGS (n=2)	Undef (n=2)
Hematuria	0/34	2/2	1/2
Hypertension	0/34	2/2	1/2
Renal insuff	0/34	2/2	0/2
Positive IFL	11/34	0/2	1/2

34) In these cases, light microscopy revealed no or little structural changes including a slight increase of the mesangial matrix and/or evidence of focal thickening of the glomerular capillary basement membranes Fifty three % of the subjects were boys

2 Focal segmental glomerulosclerosis (FSGS) (n=2) Some of the glomeruli were sclerosed while the remainder were normal

3 Undefined findings (Undef) (n=2) In this presumably heterogeneous structural group no definite classification could be made using accepted criteria Borderline abnormalities were present and consisted of slight hypercellularity of the glomeruli and of some of the epithelial elements No definite signs of tubular lesions crescent formations or sclerosed glomeruli were found In this group the mean time from clinical diagnosis to biopsy was only about two months

All children were treated with steroids (prednisolone 2–2 5 mg/kg in divided doses) after the diagnosis of the nephrotic syndrome was made It was gradually reduced after the urine became protein free for at least one week

The patients were divided into four groups based on the response to steroids

Group a No relapse or infrequent relapses (n=8) These patients responded initially to steroid therapy by becoming protein free (steroid sensitive) and had less than two relapses in twelve months

Group b Frequent relapses (n=13) These patients were initially steroid sensitive but had a greater number of relapses than patients in group a)

Group c Steroid-dependent (n=9) These patients developed proteinuria again when the dose of prednisolone was reduced to less than about 10 mg/day

Group d Steroid refractory (n=8) These patients did not respond to a high steroid dose during the first month of treatment Early non responders did not respond to initial steroid therapy

Patients in groups *b c* and *d* were given cytotoxic therapy as either cyclophosphamide (3–5 mg/day for a period of 6–12 weeks) or chlorambucil (0 1–0 2 mg/day for a period of 6–12 weeks) Twenty five courses of therapy were administered to a total of 23 children This treatment was combined with prednisolone which was given either from the beginning or, in three cases later during the course

RESULTS

In patients with the idiopathic nephrotic syndrome with little or no structural changes (MCNS), clinical symptoms, such as hematuria, hypertension or elevated urea values did not persist (Table 1) During the early acute phase transient microscopic hematuria was present in one patient and a moderately elevated blood pressure was present in four patients In the two groups of patients with evidence of focal glomerulosclerosis (FSGS) or atypical undefined changes (Undef) all three clinical symptoms were more common No relationship was found between clinical complications and immunofluorescent deposits

All except one of the patients who had minimal changes (MCNS) (33/34, 97%) showed an initial decrease in proteinuria in response to steroids (Table 2) Almost two-thirds of them remained steroid sensitive during the period of observation but 13 of 21 patients who continued to be steroid sensitive (62%) subsequently had multiple exacerbations of their disease (frequent relapsers) In the other two morphological categories, no patients were initially steroid sensitive (early non responders) (Table 2)

The indication for cytotoxic therapy was based on the previous response to steroids and the clinical course Totally 23 patients were given cytotoxic therapy, i e , 19 of 34 patients with MCNS and all 4 patients with FSGS or Undef (Table 2) In all 4 patients with FSGS or Undef and in the early non responder with MCNS, the proteinuria showed little or no response to this therapy Their urinary excretion of protein did not fall below 50 mg/kg/day In the remaining 18 patients, all with MCNS the response to cytotoxic therapy was good (Table 3) Of these 18 patients, 12 remained in remission after 10 to 96 months of observation, with a mean period of 45 months

Thirteen patients with MCNS who were steroid dependent or refractory were given cytotoxic drugs (Table 3) Only 5 of them have

Table 2 *Distribution of patients, according to renal morphology, showing clinical course and steroid response before cytotoxic therapy*

	MCNS (n 34)			FSG (n=2)			Undef (n=2)		
	Total no	Cyclo-phosha mide	Chlor ambucil	Total no	Cyclo-phospha mide	Chlor ambucil	Total no	Cyclo-phospha mide	Chlor ambucil
No relaps or infrequent relapses	8	–	–	–	–	–	–	–	–
Frequent relapses	13	4	2	–	–	–	–	–	–
Steroid dependent	9	7	2	–	–	–	–	–	–
Steroid refractory	4[a]	3	1[b]	2[c]	2	0	2[c]	1	1[b]

[a] One patient was early non responder
[b] Patient was previously given cyclophosphamide (not shown in table)
[c] Both patients were early non responders

relapsed, i e , four have again become steroid sensitive (responders) and one has remained steroid refractory Some clinical data concerning patients in the MCNS group who were treated with cytotoxics are given in Table 4 After therapy the mean number of relapses decreased significantly during follow up It is to be noticed that most children with steroid dependent and refractory nephrotic syndrome had previously had frequent relapses

DISCUSSION

In the present retrospective study of the idiopathic nephrotic syndrome in children, about 89% of the cases had little or no evidence of glomerular lesions (MCNS) on biopsy The reported figure for MCNS in a larger material is 75–85% (16, 18, 26) The proportion of patients with focal segmental glomerulosclerosis (FSGS) (5%) was comparatively low (3, 26) The two patients whose renal biopsy specimens could not be classified (Undef) require further investigation and follow-up before a definite diagnosis can be made Some cases have been described in which the renal morphology was never defined or at least not until further biopsies were performed In one report about 10% of the patients with focal glomerulosclerosis (13) could not be diagnosed on

Table 3 *Number of initial remissions and steroid response after cytotoxic therapy in patients with minimal change nephrotic syndrome (MCNS)*

		After cytotoxic therapy				
Before cytotoxic therapy	Total no	In remission (duration months)	*Infrequent relapses*	*Frequent relapses*	*Steroid dependent*	*Steroid refractory*
Frequent relapses						
Cyclophosphamide	4	3 (42–78)	1	–	–	–
Chlorambucil	2	1 (11)	–	1	–	–
Steroid dependent						
Cyclophosphamide	7	5 (24–96)	1	1	–	–
Chlorambucil	2	1 (10)	1	–	–	–
Steroid refractory						
Cyclophosphamide	3	1 (58)	–	1	–	1
Chlorambucil	1	1[a] (10)	–	–	–	–

[a] Before treatment with chlorambucil this patient was given cyclophosphamide (not shown in table) and after this he again became steroid refractory

Table 4. *Clinical data in children with minimal change nephrotic syndrome (MCNS) selected for cytotoxic therapy*

Clinical course before cytotoxic therapy	Age at onset (months)	Follow up time (months)		Average no of relapses per patient per 180 days	
		Before cytostatics	After cytostatics	Before cytostatics	After cytostatics
Frequent relapses	60	8	10	3 0	1 2
	54	7	78	1 8	0
	60	18	61	1 2	0
	62	58	11	2 2	0
	34	62	36	1 7	0 5
	26	29	42	1 3	0
Mean	49 3	30 3	39 7	1 87	0 28
Steroid dependent	20	88	27	1 1	0
	18	5	26	4 8	0 8
	45	26	60	0 8	0
	27	18	96	1 5	0
	52	6	66	2 0	1 6
	20	36	62	0 3	0
	18	35	32	1 0	0 4
	48	9	24	3 1	0
	155	26	10	1 8	0
Mean	44 8	27 7	44 8	1 82	0 31
Steroid refractory	22	13	65	(Persistent proteinuria)	
	16	36	72	1 8	1 0
	16	11	58	0 9	0
	26	10	10	3 0	0
Mean	20 0	17 5	51 3	1 90	0 33

the first biopsy In the present study, the two patients with biopsy findings that could not be definitely classified (Undef) and the two with FSGS showed a steroid response that was found in only one of thirty-four patients with MCNS (early non-responders) The histopathological diagnosis should therefore be taken into consideration when making a prognosis

Following cytotoxic therapy, regardless of indications, the majority of children with MCNS (63%) remain in remission after an average observation time of 45 months In accordance with several previous studies this therapy in combination with steroids seems more effective than steroids alone in inducing a lasting remission in frequent relapsing children (5, 7, 8, 14, 17) As most of the steroid dependent and refractory patients had had frequent relapses previously, these results support the view that combined therapy seems to decrease the rate of subsequent relapses during the first years after onset of the disease The effect of cytotoxic drugs alone was not assessed Previous observations suggest that cyclophosphamide together with steroids induce longer remissions than cyclophosphamide alone (17, 20) In the three patients in this study who were initially given only cytotoxics, two continued to have proteinuria until steroids were added

Several patients again became sensitive to steroids after treatment with cytotoxic drugs was initiated, a finding which has also been noted in earlier reports (5, 25) It is not certain whether this change is due to a spontaneous variation in steroid responsiveness (19) or to a specific cytotoxic effect on the

basic mechanism of the glomerular lesion (5) or to some other mechanism

Of five (13%) initially steroid refractory patients (early non-responders) none became free of proteinuria after steroids and cytotoxic drugs Recent studies are reported to have been more successful in this group of patients (17, 25). About half of the early non-responders in these studies were subsequently found to be free of proteinuria regardless of whether they had MCNS or some other histological diagnosis The reason for this discrepancy in results is probably certain features of the individual disease rather than the therapy, which in some cases was about the same as regards dose and duration

The present material does not permit statistical evaluation of the choice of cytotoxic drug (cyclophosphamide or chlorambucil) or the duration of this therapy Cameron et al (7) found no convincing relationship between the duration of cyclophosphamide treatment and the stability of remission and stated that treatment for more than eight weeks could not be recommended

The decision concerning the use of cytotoxic drugs in addition to steroids in the treatment of children with the idiopathic nephrotic syndrome must rest upon the balance between therapeutic efficiency and toxicity of the two agents One severe risk of cyclophosphamide and chlorambucil is the permanent suppression of reproductive cells (12, 15, 22) It appears that the duration of administration correlates with the severity of the damage The gonads have been affected in prepuberal boys given cyclophosphamide courses for more than thirteen weeks (12) Even a six-week course of therapy may have toxic effects. Recovery of reproductive function has been reported in adult men (23) In selected nephrotic children with histological evidence of minimal change lesions and with resistance to conventional doses of steroids or unacceptable side effects of steroids, there may be justification for using cytotoxic therapy It should not, however, be used as the first drug of choice.

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Submitted June 12, 1979
Accepted Oct 2, 1979

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Acta Pædiatr Scand 69 321–325, 1980

DEVELOPMENTAL ASPECTS OF GASTRIC INHIBITORY POLYPEPTIDE (GIP) AND ITS POSSIBLE ROLE IN THE ENTEROINSULAR AXIS IN NEONATES

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ABSTRACT Lucas, A, Sarson, D L, Bloom, S R. and Aynsley Green, A (University Department of Paediatrics, John Radcliffe Hospital, Oxford and Hammersmith Hospital, London, England) Developmental aspects of gastric inhibitory polypeptide (GIP) and its possible role in the enteroinsular axis in neonates Acta Paediatr Scand, 69 321, 1980.—Little is known on the development of the release of gastric inhibitory polypeptide (GIP) in neonates or on its potential role in the enteroinsular axis Using cross-section data collection we studied (a) 100 preterm neonates either at birth (cord blood), or before or after a feed on the sixth or 24th day and (b) 63 term neonates at birth or on the sixth day Blood samples were assayed for GIP, insulin and glucose At birth plasma GIP concentrations were low compared with fasting adults ($p<0.01$) Basal plasma levels were significantly higher at six days in fed infants, but not in a group of sick preterm infants who had never been fed orally On sixth day there was no GIP response to a feed, but by 24th day there was a marked postprandial elevation ($p<0.01$) In preterm infants the insulin response was 68% greater at 24 days than at six days in spite of a similar glycaemic response We hypothesize that this increasing postnatal insulin response to enteral feeding may be due to the commencement of the postprandial release of GIP thought to be an important effector in the enteroinsular axis

KEY WORDS Neonates, gastric inhibitory polypeptide, insulin, glucose, enteroinsular axis

The term enteroinsular axis was coined by Unger in 1969 to describe the transmission of signals from the gut to the pancreatic islets (19) These signals account for the enhanced insulin response in adult man to oral, as opposed to intravenous glucose (15) The enteroinsular axis is complex and may involve both neural factors and insulinogenic endocrine transmitters from the gut mucosa ('incretin') (9) The strongest "incretin" candidate at present is the gut hormone gastric inhibitory polypeptide (GIP) (7, 8 17), though studies with GIP antibodies suggest that other incretin factors have yet to be discovered (7) Little is known of the enteroinsular axis in the neonatal period, but in a recent study, King et al were not able to demonstrate gastrointestinal enhancement of the insulin response to glucose during the first few days of life (11) We have shown, however, that at the time of the first feed at 3–6 hours of age, no GIP response occurred following a 10% dextrose or milk feed (13, 4), which may be related to the absence of enhanced insulin release following oral stimuli noted by King et al There is no previous information on the development of GIP release in neonates or indeed on the time course of the postnatal development of an effective enteroinsular axis Such information might throw light on whether the progressive postnatal increase in insulin release (12), and increasing insulin response to oral glucose in neonates (16) could, in part, be related to the

We have examined developmental aspects of the release of GIP, glucose and insulin following a feed of human milk We also report on GIP levels in a group of 63 term infants, studied for a more limited period

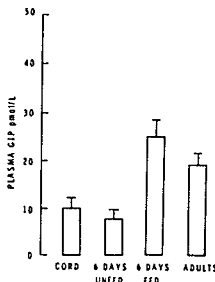


Fig 1 Plasma GIP concentration (pmol/l ± S E M) in preterm infants at birth (n=6, venous cord blood) and basal GIP concentrations on the sixth day, fed (n=10) and unfed (n=10), compared with the levels seen in healthy fasting adults (n=12)

PATIENTS AND METHODS

Sixty three term infants (mean birth weight 3290±30 g SEM) and 100 preterm infants (mean birth weight 1970±30 g mean gestation 33 5±0 1 weeks) were studied with the approval of the ethics committee Each infant contributed *only one* venous blood sample which was taken either at birth (venous cord blood taken at normal vaginal delivery) or postnatally at the same time as blood was also required for routine clinical monitoring purposes

Preterm infants were sampled either at birth (n=6) or at one of two postnatal ages, six or 24 days, when blood was taken either before or at 30 60 or 120 min after the beginning of a feed of pooled human milk given via a nasogastric tube over five minutes The infants were nursed and fed, as described previously (Lucas et al (14)) The mean feed volumes at six days and 24 days were similar 21 5 and 22 5 ml/kg respectively 8–13 samples were taken at each time period

In addition, a group of ten preterm infants (mean birth weight 1875±243 mean gestation 33±1 weeks) who had, in accordance with the policy of the Unit received 5% dextrose intravenously since birth on account of severe hyaline membrane disease were also studied

Term infants were sampled either at birth (n=20) or on the sixth day of age at the time of the routine Guthrie test Samples were taken either just before a formula (Cow and Gate premium) feed (mean duration 30 min mean inter feed interval 4 hours) or at 55, 90 or 150 min after the start of the feed The mean feed volume, determined by measuring in a sample of 33 infants was 20 ml per kg 8–12 blood samples were taken at each sampling period In addition, plasma GIP concentrations were measured in twelve healthy fasting adults

Venous samples were collected into cooled tubes containing 2000 KIU of aprotonin (Trasylol, Bayer) Cord plasma and postnatal samples were assayed for GIP as described previously (18) using a sensitive and specific radioimmunoassay capable of detecting changes between adjacent samples of 5 pmol/l The antibody used at a final dilution of 1 96000, showed negligible cross reactivity with any other gut hormone In addition, postnatal samples from preterm infants were assayed for blood glucose using the enzymatic methods described by Bergermeyer (5) and insulin by the radioimmunoassay described by Albano (2)

Statistical analyses were performed using the non-parametric Mann Whitney Rank Sum Test, but for ease of comparison the results are expressed as means and standard errors

RESULTS

GIP

In *preterm infants* cord plasma GIP concentrations were 10±2 pmol (mean ± S E M), significantly lower than the level in healthy fasting adults, 19±2 pmol/l (p<0 01) (Fig 1) The group of infants who had not been fed orally for six days after birth had persistently low plasma GIP concentrations of 8±5 pmol/l, Fig 1 In the fed group of sixth-day infants, however, basal levels of GIP were significantly higher (25±4,

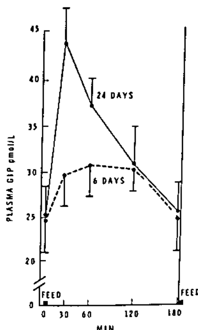


Fig 2 Plasma GIP concentration (pmol/l ± S E M) before and after a feed of human milk on the sixth day (dotted line) and 24th day (continuous line) 8–13 samples were taken at each time period each from a different neonate

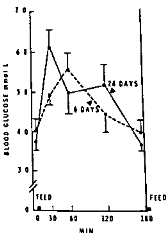


Fig 3 Blood glucose concentration (mmol/l ± S E M) before and after a feed of human milk on the sixth day (dotted line) and 24th day (continuous line) 8–13 samples were taken at each time period each from a different neonate

$p<0\ 01$), but levels did not change following a feed (Fig 2) In contrast, the 24-day old group showed a marked rise in GIP from 26±4 pmol/l just prior to the feed to 43±4 pmol/l at 30 min ($p<0\ 01$), Fig 2

Term infants had the same cord plasma GIP levels as preterm infants (10±2 pmol/l) and by the sixth day the level had risen significantly to 19±3 pmol ($p<0\ 02$) There was no change in GIP following a feed

Glucose

Six-day old preterm infants had a significant postprandial rise in blood glucose from a basal level of 4 0±0 3 mmol/l to 5 6±0 4 at 60 min ($p<0\ 005$), Fig 3 The 24-day group had a more prolonged elevation of glucose from the basallevelof3 7±0 2mmol/lto6 1±0 5at30min ($p<0\ 001$) and to 5 3±0 4 at 120 min ($p<0\ 005$), Fig 3 In spite of the difference in pattern of glucose response, the area under the glucose curve between two successive feeds was very similar at the two postnatal ages (only 7% greater in the 24-day group)

Insulin

In the six-day group of preterm infants plasma insulin rose from a basal value of 23±4 pmol/l to 203±36 at 30 min ($p<0\ 001$), but then fell steeply towards basal values (Fig 4). In contrast, at 24 days the insulin rise, from a basal level of 25±3, was more sustained reaching 276±50at30min($p<0\ 001$)and268±46at60min ($p<0\ 001$), the latter level being significantly higher than the 60-min value in the six-day group ($p<0\ 02$) The area under the response curve between successive feeds was 68% greater in the 24-day group than the six-day group (Fig. 4)

DISCUSSION

We have demonstrated marked developmental changes in the postnatal release of gastric inhibitory polypeptide (GIP) At birth plasma GIP concentrations were half of the fasting adult value, but by six days of life basal levels in term and preterm infants had doubled In contrast, however, in the group of six-day-old infants who had never been fed orally, and were maintained on a constant 5% dextrose infusion intravenously, plasma GIP concentra-

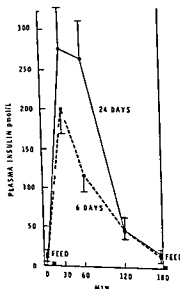


Fig 4 Plasma insulin concentration (pmol/l ± S E M) before and after a feed of human milk on the sixth day (dotted line) and 24th day (continuous line) 8–13 samples were taken at each time period, each from a different neonate

tions were low and similar to the plasma levels at birth, suggesting that the postnatal elevation of the basal concentration in healthy fed infants may have been induced by enteral feeding However, the presence of hyaline membrane disease in these unfed infants complicates the interpretation of our findings

On the sixth day, healthy term and preterm infants showed no GIP response to feed of human milk, whereas at 24 days of age in preterm infants (whom we were able to study further into the neonatal period than term ones) there was a substantial postprandial elevation 30 minutes after the start of the feed The rise in plasma GIP observed at this age is comparable to the rise observed by Besterman et al (6), in healthy adults following a mixed meal

We have considered the possibility that the development of GIP responsiveness to oral feeding may play a role in the development of an enteroinsular axis in human neonates In this study preterm infants were three hourly fed with a similar feed bolus on the sixth and the 24th day The glycaemic response, as judged by the area under the response curve, between successive feeds was very similar at those two postnatal ages, though the pattern of release was slightly different (Fig 3) In contrast, the insulin response was significantly more prolonged in the 24 day group and the area under the response curve 68% greater than that at six days It is possible that the enhanced insulin release in the 24 day group was related to the postprandial release of GIP at this age resulting in a greater degree of islet cell stimulation This argument is consistent with our observation that the GIP elevation preceded the time period when the insulin response was greater than that in the six day group (60 min)

The relationships between insulin glucose and GIP are likely to be complex For example, the observation that the glucose response to a feed at six and 24 days were similar in spite of an enhanced insulin release might imply that there had been increasing insulin resistance during this period, since the greater insulin release at 24 days should have resulted in more rapid glucose disposal However a more likely explanation for the similar glucose responses is that an insulin stimulated increased glucose utilisation in the 24-day group may have been balanced by increased entry of glucose into the blood which may result from improved digestion and absorption of lactose at this age (3) and, in addition, perhaps because of improved gluconeogenesis

The possible effects of oral feeding itself on the development of the enteroinsular axis is of interest In animals, feeding seems to be an important factor in stimulating postnatal development of the insulin response to parenteral glucose in rats (10) and piglets (1) These findings have been interpreted in terms of β cell maturation, but a possible alternative hypothesis would be that previous feeding as suggested by our observations, had increased circulating (basal) GIP levels, which would be expected to potentiate the glucose induced insulin release It is also possible that if feeding does induce β cell maturation, the trophic factor might be GIP itself

Clearly, further studies are needed to evaluate the role of the enteroinsular axis in the neonate, since this information may explain some of the important developmental chan in intermediary metabolism which occur in early weeks of life

ACKNOWLEDGEMENT

A L was in receipt of a Wellcome Research Fellowsh

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Submitted June 14 1979
Accepted Nov 9 1979

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Acta Pædiatr Scand 69 327–330, 1980

PYRIDOXAL PHOSPHATE CONCENTRATION IN BLOOD IN NEWBORN INFANTS AND THEIR MOTHERS COMPARED WITH THE AMOUNT OF EXTRA PYRIDOXOL TAKEN DURING PREGNANCY AND BREAST FEEDING

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ABSTRACT Ejderhamn J and Hamfelt A (Departments of Paediatrics and Clinical Chemistry, Sundsvalls Hospital, Sundsvall, Sweden) Pyridoxal phosphate concentration in blood in newborn infants and their mothers compared with the amount of extra pyridoxol taken during pregnancy and breast feeding Acta Paediatr Scand, 69 327, 1980.—The concentrations of pyridoxal phosphate have been estimated in cord blood and capillary blood samples taken at 3 hours, 2 days, 4 days, 7 days and 6 weeks of age, from eleven full term infants Pyridoxal phosphate concentrations were also determined in venous blood samples taken from the mothers at delivery A highly significant correlation between pyridoxal phosphate in cord whole blood and venous whole blood taken from the mothers at delivery was found Infants whose mothers had taken extra pyridoxol during pregnancy had a higher concentration of pyridoxal phosphate at 3 hours of age compared with infants whose mothers had not taken extra pyridoxol During the first week of life the concentration of pyridoxal phosphate in capillary blood decreases strikingly At 6 weeks of age the concentration of pyridoxal phosphate is in the same range as that of normal adults Findings are also discussed which indicates that 1) Vitamin B_6 is transported in breast milk 2) The giving of supplemental pyridoxol during pregnancy in ordinary doses (2–6 mg/day) does not have an antilactogenic effect No correlation between the erythrocyte aspartate aminotransferase activation with pyridoxal phosphate in vitro and pyridoxal phosphate concentration in plasma was found during the first 6 weeks of life

KEY WORDS Pyridoxal phosphate concentration cord blood capillary blood, venous blood, newborn infants, mothers

It has been shown from earlier studies (1, 6 11) that the concentration of pyridoxal phosphate in cord blood is much higher than in the venous blood from the mother Some studies indicate that the concentration of pyridoxal phosphate is at the same level in the capillary blood of the newborn infant as in cord blood (1) No studies of the normal reference values of pyridoxal phosphate during the neonatal period seem to have been performed In this study pyridoxal phosphate concentrations in infants up to the age of 6 weeks as well as in their mothers have been followed

MATERIAL

Pyridoxal phosphate concentration in whole blood was determined in both capillary and cord blood samples of 11 full term infants The mothers of the infants were enlisted from those who delivered on a Monday morning because it made blood sampling easier to organize We chose mothers who were healthy during pregnancy and who had uncomplicated deliveries at the expected time At delivery the mothers were asked for permission for blood sampling to be carried out

The age of the mothers varied from 17 to 30 years After delivery the mothers were interviewed about their oral consumption of daily extra vitamin B_6 taken during pregnancy This varied between 0–6 mg/day and had been started in the 3rd 4th or 5th month of pregnancy The infants were examined by specialists in paediatrics during their stay in hospital and were found to be healthy Capillary blood samples were taken from the infants at 3 hours of age and on the 2nd and 4th days of life On the 7th day of life capillary blood samples were taken from 2 of

Abbreviations Py 5 P=pyridoxal phosphate EVF=erythrocyte volume fraction Vitamin B_6=pyridoxol pyridoxal pyridoxamin

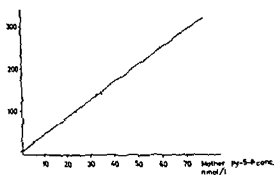


Fig 1 Pyridoxal phosphate concentration in cord whole blood in relation to pyridoxal phosphate concentration in venous whole blood of the mothers at delivery A linear regression line of y = 4 00X + 9 11 with a correlation coefficient of r = 0 9752 was found

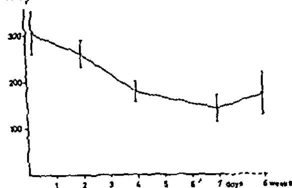


Fig 3 Pyridoxal phosphate concentration in capillary blood during the first 6 weeks of life, corrected for EVF-variations The 6 week-values of infants no 5 and no 10 are excluded from the curve (see *Discussion*)

tions in whole blood until the sixth week of life and in blood corrected for EVF-variations are shown in Figs 2 and 3 (The 3 hour- and 4th day-values of case 2 are missing) No correlation between the erythrocyte aspartate amino transferase activation with pyridoxal phosphate in vitro and pyridoxal phosphate concentration in plasma were found during the first 6 weeks of life Duration of breast feeding is shown in Table 3 The mothers were divided into two groups depending on whether or not they had recieved extra pyridoxol during pregnancy

DISCUSSION

It can be concluded from Table 1 that infants whose mothers had taken extra pyridoxol during pregnancy had higher concentrations of pyridoxal phosphate in blood when compared with infants whose mothers had not taken supplemental pyridoxol. Similar results from cord blood have been reported earlier (6, 9) but in this study it has been shown even to apply to capillary blood

Table 2 shows that all infants whose mothers had taken extra pyridoxol during pregnancy (with the exception of infant no. 5)

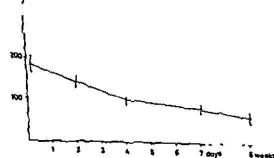


Fig 2 Pyridoxal phosphate concentration in capillary blood during the first weeks of life The 6 week values of infants no 5 and no 10 are excluded from the curve (see Discussion)

Table 3 *Duration of breast feeding*

Mothers with extra pyridoxol intake		Mothers without extra pyridoxol intake	
Case no	Months	Case no	Months
1	10	3	6
2	2	6	4
4	–*	7	3 5
5	2	9	4
8	7 5		
10	2		
11	3		
M	≈4 4		≈4 37
N	6		4

* Result not available

Table 1. *Concentration of pyridoxal phosphate in capillary blood of infants at three hours of age and in venous blood of the mothers at delivery*

Mothers with extra pyridoxol intake			Mothers without extra pyridoxol intake		
Blood pyridoxal phosphate conc nmol/l			Blood pyridoxal phosphate conc nmol/l		
Case no	Mother	Infant	Case no	Mother	Infant
1	–[a]	196	3	–[a]	154
2	–[a]	–[a]	6	33	132
4	–[a]	286	7	35	147
5	32	129	9	35	113
8	24	183			
10	69	260			
11	54	249			
M	45	217		34	137
V	4	6		3	4

[a] Results not available

the infants Capillary blood samples were taken from 7 infants at 6 weeks of age Venous blood samples for determination of pyridoxal phosphate were taken from 7 mothers at delivery

METHODS

Blood sampling

1–2 ml capillary blood was drawn into a 2 ml vacutainer tube containing 50 μl MgK EDTA The tube was turned 10 times to mix the EDTA solution with the blood The cord blood and venous blood samples were also collected in a tube of vacutainer EDTA The blood samples were stored in a deep freeze at −20°C until analyzed

Pyridoxal phosphate concentration in blood

Pyridoxal phosphate concentrations were determined in cord, venous and capillary blood samples according to a modification of a method previously described (5 7) (The standard deviation of 53 double determinations was 3 35 at a mean value of 141 nmol/l)

Aspartate amino transferase (E C 2 6 1 1) in blood

Aspartate amino transferase in blood was determined with and without activation with pyridoxal phosphate according to the recommendations of the Scandinavian Enzyme Committee (10)

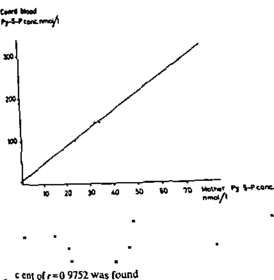
RESULTS

Pyridoxal phosphate concentrations in blood samples taken at 3 hours of age were determined in two groups of infants, as shown in Table 1 In the first group the mothers had taken extra pyridoxol whereas in the second group no supplemental pyridoxol had been given Table 1 also shows the mothers concentration of pyridoxal phosphate in venous blood taken at delivery Pyridoxal phosphate concentrations in blood samples in infants and mothers compared with the amount of extra pyridoxol taken during pregnancy are presented in Table 2 The correlation between pyridoxal phosphate concentrations in cord whole blood and venous whole blood taken from the mothers at delivery is shown in Fig 1 The pyridoxal phosphate concentra

Table 2 *Concentration of pyridoxal phosphate in infants and mothers compared to pyridoxol consumption during pregnancy*

Case no	Birth weight (g)	Weeks of gestation	The mothers daily extra pyridoxol consumption during pregnancy (mg)	Py 5 P conc in the mothers venous blood at delivery (nmol/l)	Py 5 P conc in capillary blood at 3 hours of age (nmol/l)	Py 5 P conc in capillary blood at 6 weeks of age (nmol/l)
1	3 430	38	2	–[a]	196	–[a]
2	4 190	40	2	–[a]	–[a]	–[a]
3	3 800	41	0	–[a]	154	–[a]
4	3 000	39	2	–[a]	286	–[a]
5	2 690	42	4	32	129	280
6	3 300	39	0	33	132	50
7	4 830	41	0	35	147	64
8	4 730	38	2	24	183	111
9	3 940	42	0	35	113	50
10	3 300	40	>6	69	260	248
11	2 950	40	4	54	249	54

[a] Results not available



c ent of r = 0 9752 was found

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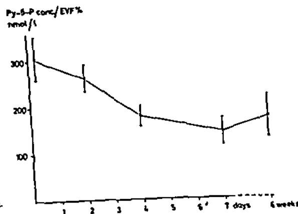


Fig 3 Pyridoxal phosphate concentration in capillary blood during the first 6 weeks of life corrected for EVF-variations The 6 week values of infants no 5 and no 10 are excluded from the curve (see Discussion)

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It can be concluded from Table 1 that infants whose mothers had taken extra pyridoxol during pregnancy had higher concentrations of pyridoxal phosphate in blood when compared with infants whose mothers had not taken supplemental pyridoxol Similar results from cord blood have been reported earlier (6, 9) but in this study it has been shown even to apply to capillary blood

Table 2 shows that all infants whose mothers had taken extra pyridoxol during pregnancy (with the exception of infant no 5)

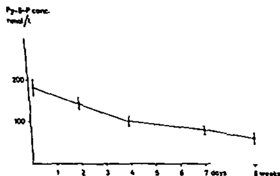


Fig 2 Pyridoxal phosphate concentration in capillary blood during the first weeks of life The 6 week values of infants no 5 and no 10 are excluded from the curve (see Discussion)

Table 3 *Duration of breast feeding*

Mothers with extra pyridoxol intake		Mothers without extra pyridoxol intake	
Case no	Months	Case no	Months
1	10	3	6
2	2	6	4
4	–*	7	3 5
5	2	9	4
8	7 5		
10	2		
11	3		
M	≈4 4		≈4 37
N	6		4

* Result not available

Table 1. *Concentration of pyridoxal phosphate in capillary blood of infants at three hours of age and in venous blood of the mothers at delivery*

Mothers with extra pyridoxol intake			Mothers without extra pyridoxol intake		
Blood pyridoxal phosphate conc , nmol/l			Blood pyridoxal phosphate conc , nmol/l		
Case no	Mother	Infant	Case no	Mother	Infant
1	–[a]	196	3	–[a]	154
2	–[a]	–[a]	6	33	132
4	–[a]	286	7	35	147
5	32	129	9	35	113
8	24	183			
10	69	260			
11	54	249			
M	45	217		34	137
N	4	6		3	4

[a] Results not available

the infants Capillary blood samples were taken from 7 infants at 6 weeks of age Venous blood samples for determination of pyridoxal phosphate were taken from 7 mothers at delivery

METHODS

Blood sampling

1–2 ml capillary blood was drawn into a 2 ml vacutainer tube containing 50 μl MgK EDTA The tube was turned 10 times to mix the EDTA solution with the blood The cord blood and venous blood samples were also collected in a tube of vacutainer EDTA The blood samples were stored in a deep freeze at −20°C until analyzed

Pyridoxal phosphate concentration in blood

Pyridoxal phosphate concentrations were determined in cord, venous and capillary blood samples according to a modification of a method previously described (5 7) (The standard deviation of 53 double determinations was 3 35 at a mean value of 141 nmol/l)

Aspartate amino transferase (E C 2 6 1 1) in blood

Aspartate amino transferase in blood was determined with and without activation with pyridoxal phosphate according to the recommendations of the Scandinavian Enzyme Committee (10)

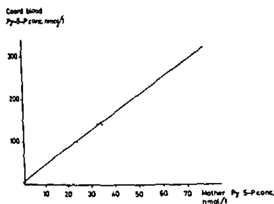
RESULTS

Pyridoxal phosphate concentrations in blood samples taken at 3 hours of age were determined in two groups of infants, as shown in Table 1 In the first group the mothers had taken extra pyridoxol whereas in the second group no supplemental pyridoxol had been given Table 1 also shows the mothers' concentration of pyridoxal phosphate in venous blood taken at delivery Pyridoxal phosphate concentrations in blood samples in infants and mothers compared with the amount of extra pyridoxol taken during pregnancy are presented in Table 2 The correlation between pyridoxal phosphate concentrations in cord whole blood and venous whole blood taken from the mothers at delivery is shown in Fig 1 The pyridoxal phosphate concentra

Table 2 *Concentration of pyridoxal phosphate in infants and mothers compared to pyridoxol consumption during pregnancy*

Case no	Birth weight (g)	Weeks of gestation	The mothers daily extra pyridoxol consumption during pregnancy (mg)	Py 5 P conc in the mothers venous blood at delivery (nmol/l)	Py 5 P conc in capillary blood at 3 hours of age (nmol/l)	Py 5 P conc in capillary blood at 6 weeks of age (nmol/l)
1	3 430	38	2	–[a]	196	–[a]
2	4 190	40	2	–[a]	–[a]	–[a]
3	3 800	41	0	–[a]	154	–[a]
4	3 000	39	2	–[a]	286	–[a]
5	2 690	42	4	32	129	280
6	3 300	39	0	33	132	50
7	4 830	41	0	35	147	64
8	4 730	38	2	24	183	111
9	3 940	42	0	35	113	50
10	3 300	40	>6	69	260	248
11	2 950	40	4	54	249	54

[a] Results not available



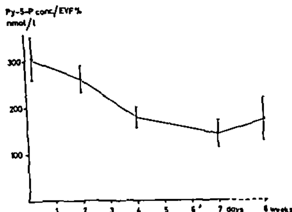


Fig 3 Pyridoxal phosphate concentration in capillary blood during the first 6 weeks of life, corrected for EVF-variations The 6 week values of infants no 5 and no 10 are excluded *from the curve (see Discussion)*

cient of r=0 9752 was found

tions in whole blood until the sixth week of life and in blood corrected for EVF-variations are shown in Figs 2 and 3 (The 3 hour- and 4th day values of case 2 are missing) No correlation between the erythrocyte aspartate amino transferase activation with pyridoxal phosphate in vitro and pyridoxal phosphate concentration in plasma were found during the first 6 weeks of life Duration of breast feeding is shown in Table 3 The mothers were divided into two groups depending on whether or not *they had recieved extra pyridoxol during preg*nancy

DISCUSSION

It can be concluded from Table 1 that infants whose mothers had taken extra pyridoxol during pregnancy had higher concentrations of pyridoxal phosphate in blood when compared with infants whose mothers had not taken supplemental pyridoxol Similar results from cord blood have been reported earlier (6, 9) but in this study it has been shown even to apply to capillary blood

Table 2 shows that all infants whose mothers had taken extra pyridoxol during pregnancy (with the exception of infant no 5)

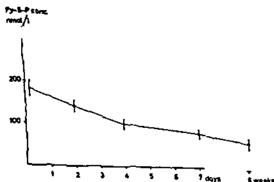


Fig 2 Pyridoxal phosphate concentration in capillary blood during the first weeks of life The 6 week values of infants no 5 and no 10 are excluded from the curve (see Discussion)

Table 3 *Duration of breast feeding*

Mothers with extra pyridoxol intake		Mothers without extra pyridoxol intake	
Case no	Months	Case no	Months
1	10	3	6
2	2	6	4
4	–[a]	7	3 5
5	2	9	4
8	7 5		
10	2		
11	3		
M	≈4 4		≈4 37
N	6		4

[a] Result not available

had very high concentrations of pyridoxal phosphate in capillary blood at 3 hours of age. In the case of infant no 5 with a 42-week gestational period and birth weight of 2 690 g, placental insufficiency could well account for the low value obtained since it is known that vitamin B_6 is actively transported across the placenta (1). At 6 weeks of age, two infants (no 5 and no 10) had very much higher concentrations of pyridoxal phosphate in blood than the other infants (Table 2). The mothers of these infants had continued to take supplemental pyridoxol for at least one month after delivery. Both mothers were breast feeding. These findings indicate that vitamin B_6 is transported via breast milk. Should extra pyridoxol therefore be continued during the breast feeding period?

A significant correlation between pyridoxal phosphate concentrations in cord whole blood and mother's venous whole blood was found and is shown in Fig 1. Earlier this has been shown in plasma samples (6, 9).

During the first week of life the concentration of pyridoxal phosphate in capillary blood decreases strikingly as seen in Fig 2. This cannot be explained by the fall of EVF values occurring during this time, as this would have resulted in an upward swing in the curve in Fig 3. At 6 weeks of age the concentrations of pyridoxal phosphate in capillary blood (Fig 2) are in the same range as in normal adults (4, 8), except for infants no 5 and no 10, mentioned earlier.

Foukas has shown (2) that pyridoxol in large doses (300–600 mg/day) has an antilactogenic effect and it has been suggested recently that giving supplemental pyridoxol in recommended doses to pregnant women inhibits the secretion of breast milk (3). The recommended daily dose of extra pyridoxol to the pregnant woman is found to be between 2 and 10 mg/day (6, 9).

In our study there is almost no difference in duration of breast feeding between the two groups of mothers (Table 3). This does not support the above mentioned danger of giving supplemental pyridoxol during pregnancy in recommended doses.

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Submitted June 27 1979
Accepted Nov 15 1979

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Acta Pædiatr Scand 69 331–335, 1980

A PROSPECTIVE STUDY OF THE EFFECTS OF CLINICALLY SEVERE PROTEIN ENERGY MALNUTRITION ON GROWTH

THERESA O SCHOLL,[1,2] F E JOHNSTON[1] J CRAVIOTO[3] and ELSA R DELICARDIE[3]

ABSTRACT School, T O, Johnston, F E, Cravioto, J C and De Licardie, E. R (Department of Anthropology, University of Pennsylvania, Philadelphia, USA) A prospective study of the effects of clinically severe protein-energy malnutrition on growth Acta Paediatr Scand, 331.—The effects of severe protein-energy malnutrition (PEM) upon the growth of children was studied in a 13-month cohort The serial records of 19 PEM children from the cohort were compared to those of cohort children with the same growth history (either failing or non-failing growth), but without severe PEM When the comparison was made on the basis of age, no differences in growth were found except for arm muscle circumference at one age Using Z-scores, comparisons were also made according to months before or after the diagnosis of PEM. When compared to children with the same growth history, PEM children showed short term differences in weight and arm muscle circumference and a mild retardation in growth in length which was not significant Any growth effects following the episode of severe PEM were accounted for by growth status preceding its onset

KEY WORDS Malnutrition, growth and development, growth failure, Mexico

Children recovering from clinically severe protein energy malnutrition (PEM) gain both weight and muscle and, after recovery, both of these variables are appropriate for their body length (2, 14) However, both weight and length are usually small for age after treatment (5, 19)

That clinical severe PEM causes long term growth retardation has been widely accepted (see e g , 12) because most children who have had PEM remain small, when followed up years later Other investigators have disagreed with this interpretation, noting that linear growth was stunted on admission This led them to suggest that many PEM children were already chronically undernourished (3, 6, 9), and that the chronic nutritional deprivation caused long term growth retardation, the clinically severe PEM having little additional effect

Each alternative is supported by studies comparing the previously malnourished either to same community controls or to their siblings (4, 5, 11, 17) However, as Richardson has pointed out, it is unlikely that these studies controlled for all factors influencing nutritional status (15), and matching done years after the onset of PEM could not have taken pre existing chronic malnutrition into account

Most sibling comparisons (8, 10, 13, 15) indicate that former patients were not significantly smaller, supporting the argument that the severe PEM did not cause any additional impairment However, sibling studies suffer from varying degrees of selective bias For example, PEM patients are excluded, 1) when they are adopted, 2) when there is no available sibling for comparison, and 3) when the available sib was also clinically undernourished In one study (10) 21% of the PEM subjects were excluded for these reasons

To date, all community and sibling studies have been retrospective, with no available data prior to hospitalization Hence they have

Table 1 *Means ($\bar{X}$) and standard deviations (S D) of weight, length, and arm muscle circumference*

Growth failure present							Growth failure absent					
No PEM			PEM				No PEM			PEM		
N	$\bar{X}$	S D	*N*	$\bar{X}$	S D	*Age* (no)	*N*	$\bar{X}$	S D	*N*	$\bar{X}$	S D
Weight (kg)												
55	2 70	0 51	14	2 63	0 37	B	192	2 85	0 39	5	2 76	0 39
58	6 41	0 79	14	6 25	1 10	6	199	6 90	0 87	5	6 62	0 78
52	7 10	0 67	14	7 10	0 98	12	168	8 11	0 92	5	7 98	0 69
52	7 82	0 71	12	7 71	1 09	18	156	9 06	0 98	5	9 44	0 82
49	8 73	0 95	12	8 25	1 28	24	151	10 25	1 00	4	10 12	0.50
48	9 81	1 10	12	9 72	1 00	30	153	11 21	1 00	4	10 83	0 76
43	10 71	1 10	11	10 72	1 30	36	154	12 15	1 10	4	11 95	1 06
Length (cm)												
55	47 9	1 6	14	47 8	1 9	B	192	48 3	2 3	5	48 7	1 8
58	62 4	2 1	14	61 6	3 5	6	199	63 5	2 3	5	63 4	2 4
52	67 6	1 7	14	67 3	3 4	12	168	70 1	2 6	5	69 7	2 8
52	71 5	2 2	12	72 2	2 7	18	156	74 9	2 9	5	75 4	3 0
49	75 0	2 7	12	75 4	2 6	24	151	79 5	2 9	4	79 9	3 0
48	79 0	3 0	12	78 5	2 9	30	153	83 4	3 2	4	83 8	3 3
43	83 0	3 0	11	81 6	1 9	36	154	87 1	3 2	4	86 0	3 2
Arm muscle circumference (cm)												
45	8 0	1 0	14	8 3	0 6	B	170	8 3	0 8	5	8 7	1 7
52	10 6	0 6	13	10 5	1 2	6	185	11 2	0 9	5	10 8	0 7
48	10 5	0 7	14	10 5	0 9	12	161	11.5	0 8	5	11 4	1 3
49	10 6	0 7	12	10 8	0 9	18	150	11 4	0 7	5	11 4	1 0
49	10 8	0 8	12	10 7	0 8	24	144	11 7	0 7	4	11 4	0 3
42	11 2	0 9	12	11 3	1 1	30	144	12 1	0 7	4	11 2	0 4
40	11 8	0 8	11	11 6	0 9	36	145	12 5	0 7	4	12 4	0 6

been unable to separate the effects of preceding chronic undernutrition from those of clinically-severe PEM In this paper, we report the results of a prospective study of this matter, utilizing longitudinal growth data taken before and after the onset of severe PEM

MATERIALS AND METHODS

The study sample consists of 276 rural Mexican children

birth and 48 months of life

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for age, along with arm muscle circumferences below a −1 S D of the expected Children manifesting this growth history were categorized as manifesting growth failure When the Harvard standards were used as reference children with growth failure were chronically underweight and stunted falling below the cut-off usually accepted a indicating chronic nutritional deprivation

During the first 38 months of their lives 19 childre

to 60 days after diagnosis One child moved away from the village after discharge from the hospital

PEM 14 of the 19 (74%

greater detail (16)

In addition to comparing PEM with and without growth failure to non PEM children of the same age comparisons were also made according to the onset of the PEM Z scores were calculated separately for children with and child with

Table 2 *Mean Z scores (Z) and standard errors (S E) for PEM children with and without growth failure, at time of diagnosis of PEM (T_0) and at monthly visits before and after diagnosis*

Growth failure present				Growth failure absent		
N	*Z*	S E	Time (mo)	*N*	*Z*	S E
Weight						
13	0 21	32	$T-3$	5	−0 18	28
13	−0 29	41	$T-2$	5	−0 31	24
13	−0 52*	31	$T-1$	5	−0 52*	26
13	−1 05**	28	T_0	5	−0 40	29
13	−1 02**	35	$T+1$	5	−1 12**	48
11	−1 08**	39	$T+2$	5	−0 81*	43
10	0 79**	33	$T+3$	5	−0 18	48
9	−0 26	46	$T+6$	5	−0 02	40
9	−0 02	41	$T+9$	5	+0 24	40
Length						
13	−0 07	39	$T-3$	5	−0 06	50
13	−0 04	48	$T-2$	5	−0 14	43
13	−0 15	44	$T-1$	5	−0 29	42
13	−0 25	47	T_0	5	−0 22	40
13	−0 21	46	$T+1$	5	−0 30	38
11	−0 13	50	$T+2$	5	−0 42	33
10	0 06	47	$T+3$	5	−0 43	32
9	−0 17	47	$T+6$	5	−0 57	49
9	0 43	46	$T+9$	5	−0 24	34
Arm muscle circumference						
13	0 16	40	$T-3$	5	−0 54	49
13	−0 39	44	$T\ 2$	5	−1 29*	49
13	−0 59*	43	$T\ 1$	5	−0 94	74
13	−1 40**	38	T_0	5	−3 03**	77
13	−0 94**	44	$T+1$	5	−2 02*	82
11	−0 84*	49	$T+2$	5	−1 09*	56
10	−0 98*	50	$T+3$	5	−0 79	54
9	−0 59	49	$T+6$	5	−0 27	75
9	−0 14	70	$T+9$	5	+0 74	54

ure This was recorded as T_0 A score one month before diagnosis was recorded as $T\ 1$ (and calculated from 21 month means) that for one month after diagnosis as $T+1$ and so on Z scores for the 5 children with PEM but who were classed as non failing was calculated from means and S D s for non failing subjects

RESULTS

Table 1 presents the means and standard deviations of weight, length, and arm muscle circumference by chronological age, for the PEM children with and without growth failure, as well as for non PEM subjects Within each growth group, differences of PEM and non-PEM children are minimal and none are statistically significant, except for arm muscle circumference at 30 months of non growth failure subjects

At 6 months of age, failing children were 87% of the Harvard standard in weight, but fell to 75% at 21 months, maintaining this position through 36 months, they may be classed as underweight (1) Length for age indicated, for failing subjects, stunting until months, and, in later ages, reduced growth indicating moderate to severe stunting (20)

As expected, children without growth failure were closer to the standard Mean weight for age never fell below 80% and length for age dropped to a minimum, at 36 months indicated as mild stunting (91% of standard)

Table 2 presents the mean Z scores arranged according to age at diagnosis of clinical PEM from $T-3$ to $T+9$ The subject who developed PEM at 38 months was excluded as there were no subsequent measurements Those who died due to clinical PEM are included, though sample sizes fall off accordingly Other fluctuations in sample size result from the inevitable attrition as subjects drop out of the study or are not measured at a particular age In addition, a few children who were born during the period but who moved into the community after birth, have been added to the cohort

PEM children did not differ from non PEM children with the same growth history at $T-3$ and $T-2$ At $T-1$, however, a trend is noticeable and depressions of growth are indicated from T_0 through $T+3$ Children who developed clinical PEM weighed significantly less and had significantly smaller arm muscle circumferences from $T-1$ through $T+2$ months, significant differences persist for one additional month in children with growth failure The differences are greatest at T_0, the time of diagnosis, for all but weight in non failing children There, the difference is greatest at $T+1$

By $T+6$, PEM children have essentially caught up in weight and arm muscle circumference to children of the same growth history but who did not develop severe PEM At

Table 1. *Means (X̄) and standard deviations (S D) of weight, length, and arm muscle circumference*

Growth failure present							Growth failure absent					
No PEM			PEM				No PEM			PEM		
N	X̄	S D	*N*	X̄	S D	Age (mo)	*N*	X̄	S D	*N*	X̄	S D
Weight (kg)												
55	2 70	0 51	14	2 63	0 37	B	192	2 85	0 39	5	2 76	0 39
58	6 41	0 79	14	6 25	1 10	6	199	6 90	0 87	5	6 62	0 78
52	7 10	0 67	14	7 10	0 98	12	168	8 11	0 92	5	7 98	0 69
52	7 82	0 71	12	7 71	1 09	18	156	9 06	0 98	5	9 44	0 82
49	8 73	0 95	12	8 25	1 28	24	151	10 25	1 00	4	10 12	0 50
48	9 81	1 10	12	9 72	1 00	30	153	11 21	1 00	4	10 83	0 76
43	10 71	1 10	11	10 72	1 30	36	154	12 15	1 10	4	11 95	1 06
Length (cm)												
55	47 9	1 6	14	47 8	1 9	B	192	48 3	2 3	5	48 7	1 8
58	62 4	2 1	14	61 6	3 5	6	199	63 5	2 3	5	63 4	2 4
52	67 6	1 7	14	67 3	3 4	12	168	70 1	2 6	5	69 7	2 8
52	71 5	2 2	12	72 2	2 7	18	156	74 9	2 9	5	75 4	3 0
49	75 0	2 7	12	75 4	2 6	24	151	79 5	2 9	4	79 9	3 0
48	79 0	3 0	12	78 5	2 9	30	153	83 4	3 2	4	83 8	3 3
43	83 0	3 0	11	81 6	1 9	36	154	87 1	3 2	4	86 0	3 2
Arm muscle circumference (cm)												
45	8 0	1 0	14	8 3	0 6	B	170	8 3	0 8	5	8 7	1 7
52	10 6	0 6	13	10 5	1 2	6	185	11 2	0 9	5	10 8	0 7
48	10 5	0 7	14	10 5	0 9	12	161	11 5	0 8	5	11 4	1 3
49	10 6	0 7	12	10 8	0 9	18	150	11 4	0 7	5	11 4	1 0
49	10 8	0 8	12	10 7	0 8	24	144	11 7	0 7	4	11 4	0 3
42	11 2	0 9	12	11 3	1 1	30	144	12 1	0 7	4	11 2	0 4
40	11 8	0 8	11	11 6	0 9	36	145	12 5	0 7	4	12 4	0 6

been unable to separate the effects of preceding chronic undernutrition from those of clinically-severe PEM In this paper, we report the results of a prospective study of this matter, utilizing longitudinal growth data taken before and after the onset of severe PEM

MATERIALS AND METHODS

The study sample consists of 276 rural Mexican children from a 13 month cohort of births Their growth was monitored at monthly intervals as part of a longitudinal study (7), providing approximately 10 000 examinations between birth and 48 months of life

Serial g [illegible] by regres [illegible] had poor fits to the regression This was [illegible] a clear, steady, and long term fall-off in weight and length for age, along with arm muscle circumferences below a −1 S D of the expected Children manifesting this growth history were categorized as manifesting growth failure When the Harvard standards were used as reference, children with growth failure were chronically underweight and stunted falling below the cut-off usually accepted a[s] indicating chronic nutritional deprivation

During the first 38 months of their lives, 19 childre[n] from the cohort developed clinically severe PEM 12 cor[responded] to the kwashiorkor type and the other 7 were o[f] the marasmic variety Three of the children died (tw[o] kwashiorkor and one marasmus type) within a period of 1 to 60 days after diagnosis One child moved away from th[e] village after discharge from the hospital

Of the children developing PEM 14 of the 19 (74% were from the group with growth failure (The diagnosis o[f] failure had been made without the investigators knowin[g] which children had developed clinical PEM) Their failur[e] occurred an average of 6 months before the onset of clin[ical] [illegible] greater detail (16)

In addition to comparing PEM with and without growt[h] failure to non PEM children of the same age comparison[s] were also made according to the onset of the PEM Z scores were calculated separately for children with an[d] without growth failure For example if a child wit[h] growth failure developed clinical PEM at 22 months, ther[e] the Z score at that time was calculated from the 22 mont[h] mean and standard deviation of subjects with growth fai[l]

Table 2 *Mean Z-scores ($\bar{Z}$) and standard errors (S E), for PEM children with and without growth failure, at time of diagnosis of PEM (T_0) and at monthly visits before and after diagnosis*

Growth failure present			Time	Growth failure absent		
N	*Z*	S E	(mo)	*N*	*Z*	S E
Weight						
13	−0 21	32	$T-3$	5	−0 18	28
13	0 29	41	$T-2$	5	0 31	24
13	0 52*	31	$T-1$	5	0 52*	26
13	−1 05**	28	T_0	5	−0 40	29
13	−1 02**	35	$T+1$	5	−1 12**	48
11	−1 08**	39	$T+2$	5	−0 81*	43
10	−0 79**	33	$T+3$	5	−0 18	48
9	−0 26	46	$T+6$	5	−0 02	40
9	−0 02	41	$T+9$	5	+0 24	40
Length						
13	−0 07	39	$T-3$	5	0 06	50
13	−0 04	48	$T-2$	5	−0 14	43
13	−0 15	44	$T-1$	5	−0 29	42
13	−0 25	47	T_0	5	−0 22	40
13	−0 21	46	$T+1$	5	−0 30	38
11	−0 13	50	$T+2$	5	−0 42	33
10	−0 06	47	$T+3$	5	−0 43	32
9	−0 17	47	$T+6$	5	−0 57	49
9	−0 43	46	$T+9$	5	−0 24	34
Arm muscle circumference						
13	−0 16	40	$T-3$	5	−0 54	49
13	−0 39	44	$T-2$	5	−1 29*	49
13	−0 59*	43	$T-1$	5	−0 94	74
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1	−0 84*	49	$T+2$	5	−1 09*	56
0	−0 98*	50	$T+3$	5	−0 79	54
9	−0 59	49	$T+6$	5	−0 27	75
9	−0 14	70	$T+9$	5	+0 74	54

ure This was recorded as T_0 A score one month before liagnosis was recorded as $T-1$ (and calculated from 21 nonth means) that for one month after diagnosis as $T+1$ and so on Z scores for the 5 children with PEM but who were classed as non failing was calculated from means and S D s for non failing subjects

RESULTS

Table 1 presents the means and standard deviations of weight, length, and arm muscle circumference, by chronological age, for the PEM children with and without growth failure, as well as for non PEM subjects Within each growth group, differences of PEM and non-PEM children are minimal and none are statistically significant, except for arm muscle circumference at 30 months of non-growth failing subjects

At 6 months of age, failing children were at 87% of the Harvard standard in weight, but fell to 75% at 21 months, maintaining this position through 36 months, they may be classed as underweight (1) Length for age indicated, for failing subjects, stunting until 12 months, and, in later ages, reduced growth indicating moderate to severe stunting (20)

As expected, children without growth failure were closer to the standard Mean weight for age never fell below 80% and length for age dropped to a minimum, at 36 months, indicated as mild stunting (91% of standard)

Table 2 presents the mean *Z* scores arranged according to age at diagnosis of clinical PEM, from $T-3$ to $T+9$ The subject who developed PEM at 38 months was excluded as there were no subsequent measurements Those who died due to clinical PEM are included, though the sample sizes fall off accordingly Other fluctuations in sample size result from the inevitable attrition as subjects drop out of the study, or are not measured at a particular age In addition, a few children who were born during the period, but who moved into the community after birth, have been added to the cohort

PEM children did not differ from non-PEM children with the same growth history at $T-3$ and $T-2$ At $T-1$, however, a trend is noticeable and, depressions of growth are indicated from T_0 through $T+3$ Children who developed clinical PEM weighed significantly less and had significantly smaller arm muscle circumferences from $T-1$ through $T+2$ months, significant differences persist for one additional month in children with growth failure These differences are greatest at T_0, the time of diagnosis, for all but weight in non-failing children There, the difference is greatest at $T+1$.

By $T+6$, PEM children have essentially caught up in weight and arm muscle circumference to children of the same growth history, but who did not develop severe PEM At $T+6$

Table 1. *Means (X) and standard deviations (S D) of weight, length, and arm muscle circumference*

Growth failure present							Growth failure absent					
No PEM			PEM				No PEM			PEM		
N	X	S D	N	X	S D	Age (mo)	N	X	S D	*N*	X	S D
Weight (kg)												
55	2 70	0 51	14	2 63	0 37	B	192	2 85	0 39	5	2 76	0 39
58	6 41	0 79	14	6 25	1 10	6	199	6 90	0 87	5	6 62	0 78
52	7 10	0 67	14	7 10	0 98	12	168	8 11	0 92	5	7 98	0 69
52	7 82	0 71	12	7 71	1 09	18	156	9 06	0 98	5	9 44	0 82
49	8 73	0 95	12	8 25	1 28	24	151	10 25	1 00	4	10 12	0 50
48	9 81	1 10	12	9 72	1 00	30	153	11 21	1 00	4	10 83	0 76
43	10 71	1 10	11	10 72	1 30	36	154	12 15	1 10	4	11 95	1 06
Length (cm)												
55	47 9	1 6	14	47 8	1 9	B	192	48 3	2 3	5	48 7	1 8
58	62 4	2 1	14	61 6	3 5	6	199	63 5	2 3	5	63 4	2 4
52	67 6	1 7	14	67 3	3 4	12	168	70 1	2 6	5	69 7	2 8
52	71 5	2 2	12	72 2	2 7	18	156	74 9	2 9	5	75 4	3 0
49	75 0	2 7	12	75 4	2 6	24	151	79 5	2 9	4	79 9	3 0
48	79 0	3 0	12	78 5	2 9	30	153	83 4	3 2	4	83 8	3 3
43	83 0	3 0	11	81 6	1 9	36	154	87 1	3 2	4	86 0	3 2
Arm muscle circumference (cm)												
45	8 0	1 0	14	8 3	0 6	B	170	8 3	0 8	5	8 7	1 7
52	10 6	0 6	13	10 5	1 2	6	185	11 2	0 9	5	10 8	0 7
48	10 5	0 7	14	10 5	0 9	12	161	11 5	0 8	5	11 4	1 3
49	10 6	0 7	12	10 8	0 9	18	150	11 4	0 7	5	11 4	1 0
49	10 8	0 8	12	10 7	0 8	24	144	11 7	0 7	4	11 4	0 3
42	11 2	0 9	12	11 3	1 1	30	144	12 1	0 7	4	11 2	0 4
40	11 8	0 8	11	11 6	0 9	36	145	12 5	0 7	4	12 4	0 6

been unable to separate the effects of preceding chronic undernutrition from those of clinically-severe PEM In this paper, we report the results of a prospective study of this matter, utilizing longitudinal growth data taken before and after the onset of severe PEM

MATERIALS AND METHODS

The study sample consists of 276 rural Mexican children from a 13 month cohort of births Their growth was monitored at monthly intervals as part of a longitudinal study (7), providing approximately 10 000 examinations between birth and 48 months of life

Serial growth records for weight and length were fitted by regression techniques to age (16) Seventy two children had poor fits to the regression This was demonstrated by a clear, steady, and long term fall-off in weight and length for age, along with arm muscle circumferences below a −1 S D of the expected Children manifesting this growth history were categorized as manifesting growth failure When the Harvard standards were used as reference, children with growth failure were chronically underweight and stunted falling below the cut off usually accepted as indicating chronic nutritional deprivation

D ring the first 38 months of their lives 19 children

the marasmic variety Three of the

kwashiorkor and one marasmus type) within a period of 15 to 60 days after diagnosis One child moved away from the village after discharge from the hospital

of the 19 (74%)

greater detail (16)

In addition to comparing PEM with and without growth failure to non PEM children of the same age, comparisons were also made according to the onset of the PEM *Z* scores were calculated separately for children with and without growth failure For example, if a child with growth failure developed clinical PEM at 22 months then the *Z* score at that time was calculated from the 22 month mean and standard deviation of subjects with growth fail

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Submitted May 26, 1979
Accepted Oct 2, 1979

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and $T+9$ months, no significant differences in either of these two measurements exist

In contrast to the above, the mean Z-scores for length reveal no significant difference between PEM and non-PEM children with the same growth histories Although the Z-scores are all negative, the differences are quite small and none are statistically significant

DISCUSSION

Children who develop clinical PEM are not significantly different from their age-peers who do not develop PEM, when we account for the presence or absence of chronic undernutrition In other words, if comparisons are based upon chronological age, any deviation in growth of children who develop clinically severe PEM may be attributed to the existence of a preceding chronic undernutrition Chronic undernutrition here is defined on the basis of growth failure, generally accepted as a suitable criterion (18)

On the other hand, if we analyze the growth of children who develop clinical PEM according to months before and after diagnosis, significant differences do exist When we compare these subjects to controls who did not develop clinical PEM, but who had similar growth histories (failure or non failure), the PEM children were, around the time of diagnosis of the clinical condition, lighter in weight with reduced arm muscle stores However, by 3 months following diagnosis and treatment, PEM children caught up to their peers of similar growth history so that, by 9 months following diagnosis, their growth status reflected not the presence or absence of clinically severe PEM, but the presence or absence of an earlier chronic undernutrition

Thus, these data agree with existing studies which have used siblings as controls They point to the fact that environmental conditions which exist prior to the onset of clinical PEM are more significant in subsequent growth than the effects of the clinical PEM The effects of the episode of PEM are, in this sample, negligible and did not contribute significantly to growth status 9 months following diagnosis

It is of interest that, had we not taken the presence or absence of growth failure into account, our results would have agreed with those studies which identify clinical PEM as significant in limiting growth If we combine all PEM children into a single group and compare them to all children without PEM, the PEM subjects are significantly smaller and lighter than non-PEM subjects at $T+9$ The reason for this lies in the fact that 14 of 19 PEM children also had growth failure, and that 199 of the 267 non PEM children did not have growth failure Thus, the failure to take pre existing status into account would have biased the results

Thus, when nutritional status before PEM is taken into account, little additional impairment can be demonstrated which is due to the severe PEM Any growth retardation following severe malnutrition is due mainly to failure before its onset

ACKNOWLEDGMENTS

Supported in part by USPHS Grants HD–AM 08939 and HD 00719 and by the Nutrition Foundation

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Submitted May 26, 1979
Accepted Oct 2, 1979

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Acta Pædiatr Scand 69 337–340, 1980

IRON DEFICIENCY IN SICKLE CELL DISEASE

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ABSTRACT Nagaraj Rao, J and Sur, A M (Department of Paediatrics, Medical College and Hospital, Nagpur, India) Iron deficiency in sickle cell disease Acta Paediatr Scand, 69 337, 1980.—Iron studies were performed on 25 children with homozygous sickle cell disease The majority (80%) of patients had never been transfused Surprisingly, the results showed that all had low serum iron and low transferrin saturation Three children had no marrow iron stores while the rest had diminished amounts of iron This may be an important finding in view of recent efforts at fortifying common salt with iron The exact effects of iron deficiency on sickle cell disease are not known and a controlled trial is called for

KEY WORDS Sickle cell disease, iron deficiency

It has recently been reported that patients with homozygous sickle cell disease may be iron deficient (12, 14, 15) This is contrary to standard teaching on the subject (18) The possibility that iron deficiency may be as widely prevalent among homozygous sicklers in India as it is among the general population has not been investigated Theoretically several points would suggest such a possibility (*a*) the iron intake is low, (*b*) the absorption of iron is hampered by the presence of phytates in the diet and (*c*) blood transfusions are given sparingly due to lack of adequate facilities A definite answer to the question whether or not homozygous sickle cell patients are iron loaded appears particularly timely now, since it has recently been proposed (13) to fortify salt with iron This paper describes the results of such an investigation in an area where a high incidence of the sickle cell trait has been previously reported (19)

PATIENTS AND METHODS

Twenty five consecutive patients (16 male and 9 female age range 2–16 years mean 8 9) who were diagnosed as homozygous sickle cell disease were studied The diagnosis was established on the basis of the clinical presentation (2) the demonstration of *in vitro* sickling by the method of Daland & Castle (5) and paper electrophoresis of haemoglobin (3)

After obtaining informed consent a fasting sample of venous blood (7 ml) was drawn from each patient into a clean iron free heparinized tube for iron studies Plasma iron was determined by the method recommended by the Expert Panel International Committee for Standardization in Haematology (9) which was slightly modified in that the iron standard was prepared from ferric ammonium sulphate (20) Total iron binding capacity was determined by Ramsay s method (16) A needle biopsy of the bone marrow was performed from the iliac crest and stained for iron (Perl s Prussian blue reaction) by the method of Wintrobe (23). A bone marrow smear known to contain iron was simultaneously stained in the same bath to serve as a control Bone marrow smears were graded for iron content by the method of Gale et al (7) Five specimens from normal subjects were also graded similarly

RESULTS

Only 20% of the patients had previously received blood transfusions as shown in Table 1 No correlation was noted between age and

Table 1 *Transfusions previously received by patients*

Age group (y)	Not transfused	Transfused (no of units in parentheses)
0–4	2	1 (1)
5–9	10	1 (5)
10–14	7	2 (3)
15 and above	1	1 (1)
Total	20 (80%)	5 (20%)

the mean plasma iron and percentage saturation of transferrin was lower If a defect in release of stored iron is to explain the low plasma iron levels in the face of the continued presence of storage iron, then the additional factor of true overall iron deficiency will have to be postulated, to account for the depletion of marrow iron stores These considerations also suggest that when sickle cell disease and iron deficiency occur together then sickle cell disease masks the iron deficiency to some extent This contention is also supported by Powars' experience with 8 patients of sickle cell disease and concomitant severe nutritionally derived anaemia (15) Therapy with iron improved the haemoglobin and brought it upto (but not more than) 70 g/l which appears to be the level of spontaneous stabilisation

In a very recent study (14) on sickle cell disease Peterson et al reported fairly similar findings in 11 out of 43 patients of sickle cell disease who did not have stainable iron in the bone marrow The serum ferritin levels were raised markedly, the mean level in this group was 533±174 μg/l In 28 patients with stainable iron in the marrow the mean serum ferritin level was 749±204 μg/l Though this difference was significant at the 5% level, both the values are raised compared to the normal of 60±3 μg/l

With this data Peterson et al do not conclude that their 11 marrow iron negative patients are truly iron deficient—though, they adduce evidence to show that these patients differ in their response to cyanate from the marrow iron positive patients With the seemingly anomalous elevation of serum ferritin to explain the authors conclude that their patients had what appears to be an excess of iron in the whole body and that there was a difficulty with iron transport'

Serum ferritin may be falsely raised in liver disease (11, 17) neoplasms (11) and in states with increased red cell turnover (11) This could explain the elevated serum ferritin levels Theoretically it could be argued that the patients in Peterson's marrow iron negative group had about 4 0 g of storage iron (on the basis that 1 μg/l of serum ferritin represents 8 mg of storage iron (22)) How this can be compatible with an absence of marrow iron stores is not easily explained unless one postulates a non specific rise of serum ferritin

It is true that bone marrow iron is not a reliable indicator of true iron stores in the young child between 6 months and 3 years (25) In our series only 6 of 25 were less than 5 years old, all the 3 children who had no bone marrow iron were above 12 years of age

We therefore conclude that our patients far from being iron overloaded are in fact iron deficient However, the exact interactions between iron deficiency and sickle cell disease are unsettled issues (8, 10) Theoretical considerations suggest that for a given concentration of hemoglobin in the erythrocyte the proportion contributed by HbS determines the susceptibility to sickling (8) and that rendering the cell somewhat microcytic makes it less prone to sickling The logical extension of this is that iron deficiency might improve the clinical status of sickle cell patients and that giving iron by increasing the blood haemoglobin concentration might actually increase the episodes of sickling (14) This is not in keeping with the experience of Powars (15) Since the incidence of iron deficiency among sickle cell patients appears to be high as shown by our study, the question whether or not to give iron is not merely an academic one The answer will have to await a controlled therapeutic trial

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Table 2 *Results of haematological studies*

	Patients		Normal (24)	
	Mean	S D	Range	Mean
Plasma iron (μmol/l)	10 5	1 3	12 7–35 9	21 8
TIBC (μmol/l)	57 8	6	45 7–77 8	60 8
% saturation of transferrin	17 4	6 2	20–50	35
Haemoglobin (g/l)	69	11 7		120
Reticulocyte count (%)	6 8	3 87	<1 0	

the number of transfusions received The poor transfusion rate is ascribed to the general lack of adequate facilities for blood transfusion

The values obtained (mean and standard deviation) for the plasma iron, the total iron binding capacity and the percentage saturation of transferrin are summarized in Table 2 Three patients had a plasma iron level less than 8 95 μmol/l while 8 had a percentage saturation of transferrin less than 16 Bone marrow iron studies (Table 3) showed that iron was absent in 3 of the patients and deficient in all the others

DISCUSSION

The very low transfusion rate is remarkable As many as 80% of the patients presented for the first time without ever having received a blood transfusion Age was not the factor determining whether or not the child had previously been transfused More than 70% of children over 10 years and 80% of those under 10 years had not received a transfusion In a comparable study from New York (14) 11 patients of sickle cell disease who did not have stainable iron in the bone marrow had

Table 3 *Results of bone marrow iron studies*

Grade	Patients	*Controls*
0	3 (12%)	0
1+	6 (24%)	0
2+	16 (64%)	0
3+	0	2
4+	0	3
5+ to 6+	0	0
Total	25	5

received an average of 10±4 (S E M) tra fusions each, and 28 patients who had sta able iron in the bone marrow had each ceived an average of 29±7 transfusions Qu inexplicably 2 of this latter group had not r ceived any transfusion at all The ability of o patients to survive upto and beyond the fir decade—with very few or no blood transf sions is all the more remarkable in view of th known severity of sickle cell disease in th tropics (21)

The iron status of these patients could b interpreted in the light of the low transfusio rate All the criteria for the diagnosis of laten iron deficiency (4) (plasma iron below 8 9 μmol/l percentage saturation of transferr less than 16 and no stainable iron in th bone marrow) were present in 3 patients Th other 22 patients had a lower than norma plasma iron level and percentage saturation o transferrin and a TIBC in the normal range They also had iron in the bone marrow though the amount was depleted Such a picture of diminished plasma iron in the presence of some albeit deficient iron stores in the mar row is similar in some ways to that described in a group of 88 patients by Bainton & Finch (1) This was a group with inflammatory or malignant disease who had a normocytic nor mochromic anaemia a mean plasma iron of 11 45 μmol/l, a mean transferrin saturation of 26% and elevated marrow stores The authors explain such an iron deficient erythropoiesis on the basis of a block in the release of iron from the reticuloendothelial stores (6) The situation in our patients differs in that our pa tients had depleted marrow iron stores, also

Acta Pædiatr Scand 69 341–345, 1980

THE RISK OF JAUNDICE IN GLUCOSE 6-PHOSPHATE DEHYDROGENASE DEFICIENT BABIES EXPOSED TO MENTHOL

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ABSTRACT Olowe, S A and Ransome-Kuti, O (Department of Paediatrics, College of Medicine of the University of Lagos Lagos Nigeria) The risk of jaundice in glucose-6-phosphate dehydrogenase babies exposed to menthol Acta Paediatr Scand 69 341, 1980.—A major cause of neonatal morbidity and mortality in Lagos, Nigeria, is severe neonatal jaundice seen in G-6-PD deficient babies The observation that the jaundice is more severe in out patient than in inpatient babies suggests that its cause is exogenous ' Mentholated ' powder which is commonly used in many clinics and at home to dress umbilical cords was suspected to be the offending agent. A controlled study of the effects of one of these powders was carried out on 60 consecutive G-6-PD deficient babies In 30 of them the umbilical cords were dressed daily with the powder while the remaining half who were untreated served as controls The treated babies developed statistically more significant jaundice than the controls Inability of neonates to conjugate menthol in this power is probably responsible for the jaundice developed by these G-6-PD defiment babies It is concluded that the use of menthol and/or camphor-containing commercial products on neonates be discontinued especially in communities where the incidence of G-6-PD deficiency is high as the use of such products may be contributing to the severity of neonatal jaundice

KEY WORDS G-6-PD deficiency, menthol neonatal jaundice

Severe neonatal jaundice is an important medical problem in many West African cities (5 9 12) Jaundice is the second commonest cause of death of the newborn and the most frequent cause of cerebral palsy in Lagos (13) On the neonatal unit of Lagos University Teaching Hospital (L U T H) an average of one exchange blood transfusion is done daily

Most of the babies receiving exchange blood transfusion on our Unit are usually referred from the community They are babies who have been delivered at home and those discharged home from other hospitals within 48 hours of birth (i e outpatients) Many of them are kernicteric when first seen In these babies the commonest cause of jaundice is glucose 6-phosphate dehydrogenase (G 6-PD) deficiency (13) Seventy per cent of them have the enzyme deficiency (12) Among the babies born in L U T H and observed in the hospital for the first 5 days of life (i e inpatients) severe neonatal jaundice is unusual (13) Serum bilirubin higher than 171 µmol/l is found in only 6% of the inpatients and of these only 17% have G 6-PD deficiency The commonest cause of jaundice among the inpatients is ABO incompatibility (13)

Thus in Lagos, there is a marked difference in the incidence and severity of jaundice between the inpatient and outpatient G 6-PD deficient babies Since the two groups come from the same population and environment, and have the same ethnic and genetic background, it is reasonable to suspect that an exogenous factor is responsible for the hyperbilirubinemia seen in the outpatient babies

A preliminary survey of the routine cares given to babies at home showed a basic difference from the practice in L U T H In our hospital the umbilical cords are cleaned with methylated spirit and then exposed to dry up On the other hand, in many local clinics and

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Submitted July 26 1979
Accepted Oct 31, 1979

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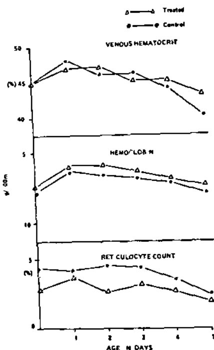


Fig 1 Mean values of daily determinations of venous hematocrit hemoglobin and reticulocyte count in mentholated powder treated and control G-6-PD deficient babies

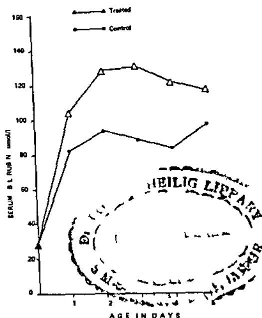


Fig 2 Mean daily serum bilirubin concentration in mentholated powder treated and control G-6-PD deficient babies

Hemoglobin hematocrit reticulocyte count and serum bilirubin (Figs 1 and 2)

Hemoglobin and venous hematocrit were similar in both groups at the start of the study After an initial rise on the first day, both variables showed a slow steady decline but there was no significant difference between the treated and control groups throughout the study Reticulocyte count in the control was slightly higher than in the treated group from the beginning to the end of the study Again, there was no significant difference between the two groups

Bilirubin concentrations exceeded 171 µmol/l in 15 of the treated babies but in only 5 of the controls The incidence of hyperbilirubinemia was significantly greater ($p<0.01$ by χ^2 technique) in the treated than in the control group

Twelve babies required phototherapy Eight of these were in the treated group The only baby whose rise of serum bilirubin could not be controlled with phototherapy was in the treated group He received one exchange blood transfusion

Bacteriology studies

Blood cultures were done on 8 treated babies and 4 of the controls All these proved sterile So too were the samples of powder sent for bacteria culture

DISCUSSION

Previous reports have shown that G 6-PD deficiency is the most important single cause of neonatal jaundice in West Africa (5, 9, 11, 13) But the pathogenesis of hyperbilirubinemia in these babies hitherto remains obscure

Our observation that, in Lagos, severe neonatal jaundice is seen more frequently in outpatient than in inpatient G 6-PD deficient babies led to an investigation of the sociocultural treatments commonly given at home to babies in this community The use of "Medicated mentholated dusting powder" was found to be a very common practice The

Table 1 *Powders used on 250 outpatient jaundiced babies*

	No of Patients	%
1 Medicated metholated dusting powder distributed by		
(a) P Z & Co Ltd	184	73 6
(b) U A C	38	15 2
2 Jonson s Baby Powder	10	4
3 Maw s Baby Powder	3	1 2
4 Boots Baby Powder	3	1 2
5 Nova Baby Powder	2	0 8
6 Local herbal preparations	10	4 0
Total	250	100%

homes, management of the umbilical cord is started by first treating it and the surrounding abdominal wall with hot fomentation This is followed by a generous application of one of the locally available proprietary powders While the whole area is still very warm, the cord, together with the powder is bandaged to the abdomen

The baby powders are often scented with various chemicals, some of which may have an adverse effect on the G 6-PD deficient red blood cells The aim of this study, therefore, was to find out if some of these powders contribute to the excessive hyperbilirubinemia seen in the G 6-PD deficient babies in Lagos

MATERIALS AND METHODS

The study was divided into two parts

Part I

In the first part 250 consecutive jaundiced *outpatient* babies requiring exchange blood transfusion on our unit were studied Their mothers were asked to produce the powders being used to dress their babies cords As a result of this preliminary exercise the most frequently used brand of powder was chosen for study

Part II

This part of the study was designed to find out if the selected powder precipitated or aggravated jaundice in G 6-PD deficient babies

The patients consisted of healthy full term babies born on our labour ward and who on examination of their umbilical cord blood were found to have G 6-PD deficiency G 6-PD activity was mesured quantitatively by the method of Capps et al (2) All premature babies and all full term babies with ABO incompatibility Rhesus isoimmunization obvious sepsis asphyxia neonatorum or congenital malformation were excluded from this study even when they also had G 6-PD deficiency Vitamin K 1 mg in the form of menadiol sodium diphosphate Synkavite was given routinely after birth to all babies Sixty consecutive G 6-PD deficient babies who fulfilled the above criteria were divided by random allocation to two

. . .

while in the remaining half the umbilical cords were cleaned but not powdered

Using standard laboratory techniques serum bilirubin hemoglobin (Hgb) hematocrit (Hct) and reticulocytes were measured on the cord bloods and then daily on the babies A blood culture was done on any baby whose serum bilirubin rose to 239 μmol/l Swabs for culture were taken from the umbilical stumps on the 3rd and 5th days of the study Bacteriological cultures were also done on samples of the powder weekly

Using Maisels guidelines (8) babies with rapidly rising serum bilirubin were treated with phototherapy or exchange blood transfusion

RESULTS

Part I

Table 1 shows that 89% of the mothers interviewed treated their babies with Medicated Mentholated Dusting Powder There were two brands of this powder The commonest one used was that distributed by Paterson Zochonis & Co Ltd This brand was therefore selected for study

Part II

Table 2 shows the clinical status of the patients The ratio of 26 males to 4 females in each group is to be expected as G 6-PD deficiency is sex linked

Table 2 *Clinical status of G-6 PD deficient babies studied*

	Treated	Control
Sex		
Male	26	24
Female	4	4
Weight (+1 S D) in kg	3 34 (+0 50)	3 28 (±0 50)
Gestation (±1 S D) in weeks	39 (+2)	39 (±1)

and excreting mechanisms of bilirubin in the perinatal period, only a small proportion of the red cell population needs to be destroyed to produce hyperbilirubinemia (10) On the other hand, it is possible that the menthol interfered with the conjugation and/or excretion of bilirubin in the treated infants

A large number of commercial products containing menthol and/or camphor are now freely available to mothers in the open market all over West Africa These products are of little therapeutic value to any baby It is hoped that the results of this investigation will alert all pediatricians to the potential *toxicity* of menthol- and/or camphor-containing commercial products, especially in those parts of world (e g *Greece, Singapore, South Africa*) where unexplained neonatal jaundice in G-6-PD deficient infants is still a serious medical problem (4, 10, 15)

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the help of Dr A Akinkugbe and Dr I Ahmed for helpful criticism Mrs S Ransome Kuti and Mrs L Olumide for technical asistance and helpful discussions and of Mrs A I Oyedele for secretarial help We also thank the chief matron and the nursing staff of wards C2 C3 and C4 for their co-operation *during the study*

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Submitted April 10 1979
Accepted Aug 10 1979

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Table 3 *Contents of "medicated mentholated" dusting powders examined*

Chemicals	Percentage Content		
	P Z & Co Ltd *	U A C (1)*	U A C (2)*
Menthol	0 5	0 5	1 0
Camphor	–	–	1 0
Kaolin lev	10	–	10
Talc B P C	49 5	37	53
Amylum	15	12 5	15
Boric acid	5	5	–
Calc carb	15	40	15
Zinc oxide	5	5	1
Total	100	100	100

* Distributed by the commercial firms P Z & Co Ltd = Paterson, Sochonis & Col Ltd , U A C = United Africa Company

powders are usually applied to the umbilical cords till they fall off and routinely as a treatment for heat rash

In this investigation, a controlled study of the effects of one such powder on G 6-PD deficient babies was carried out Apart from 1 mg Vitamin K which was given prophylactically to all babies, no other drug was administered Maternal–fetal blood group incompatibility and other common factors known to produce jaundice in the newborn period were eliminated All of them were kept under our observation in the hospital and their serum bilirubin was monitored closely The treated babies developed significantly more jaundice than the controls Our findings suggest that the use of this powder on G 6 PD deficient neonates is one of the factors contributing to hyperbilirubinemia in such infants in Lagos

An examination of these 'medicated mentholated dusting powders" shows that the only potentially toxic components are menthol and/or camphor (Table 3) Both are chemically related to naphthalene which has been reported to produce severe hemolysis in G 6-PD deficient babies (6, 14) They are readily absorbed from the mucous membrane and the skin Detoxification of these compounds occurs by hydroxylation and then conjugation with glucuronic acid by the liver (1, 3) It is reasonable, therefore, to assume that newborn infants, because of their limited conjugating capacity, are more susceptible to the toxic action of these substances Moreover, the same limitation of conjugation in the neonatal period increases the deleterious effects of hemolysis with the rapid development of kernicterus in such infants

The power used in this study contained menthol but not camphor or naphthalene Although acute hemolysis in G 6-PD deficient infants exposed to naphthalene is well known to our knowledge this is the first report associating hyperbilirubinemia in such infants with menthol We believe that this association is sufficiently strong to warrant a closer look into the use(s) of all commercial products containing menthol and/or camphor wherever neonatal jaundice occurs in G 6-PD deficient babies

However, some complex problems are raised by our observations First, babies treated with this 'mentholated' powder did not all react with jaundice It therefore seems that, in accordance with previous reports, some other factor(s), probably genetically determined (4) or metabolic (7) are essential for the development of jaundice in G 6-PD deficient babies exposed to menthol Furthermore even among the reactors, jaundice was not as severe as it is often found in the outpatient babies in Lagos This was probably due to our failure to use the powder in the same manner as parents use it at home For ethical reasons we could not foment the babies' cords in the hospital There is no doubt that absorption of menthol would be better through a warm skin with dilated blood vessels than through a skin cooled by the evaporation of methylated spirit Another important question is why there was no significant fall in Hgb and Hct or rise in reticulocyte count if menthol did indeed cause hemolysis in the jaundiced babies It is well known that these indices of hemolysis are of little value in the neonatal period (7, 15) This is because in the presence of physiologic immaturity of the conjugating

TWO YEARS FOLLOW UP OF ASTHMATIC BOYS PARTICIPATING IN A PHYSICAL ACTIVITY PROGRAMME

V GRAFF LONNEVIG S BEVEGÅRD B O ERIKSSON
S KRAEPELIEN and B SALTIN

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ABSTRACT Graff Lonnevig, V Bevegård S, Eriksson, B O, Kraepelien, S and Saltin, B (Department of Allergology, Sachs Children s Hospital and the Department of Clinical Physiology, Sodersjukhuset, Stockholm Sweden) Two years follow up of asthmatic boys participating in a physical activity programme Acta Paediatr Scand, 69 347, 1980.—Eleven boys with bronchial asthma mean age 11.2 years, participated in a 20-month long physical activity programme The training was performed in an ordinary indoor gymnasium for one hour twice a week. No drugs were given prior to the training sessions Cardiorespiratory function and dimensions and maximal aerobic capacity were determined before, during and after the training period The respiratory and circulatory dimensions increased as expected during the observation period and after correction for the influence of growth no changes were seen in the variables studied Nor did the training group differ significantly in any respect from a group of nine asthmatic boys not trained There was only a slight but not significant increase in the maximal oxygen uptake and the ventilatory coefficient in the training group as compared to the non training group After a one-week winter camp with high exercise intensity there was a rise in the total ventilation, the ventilatory coefficient and the maximal work performed but the maximal oxygen uptake was not affected All boys showed a very good ability to participate in the physical activity programme at approximately the same level as the physical education given at school

KEY WORDS Bronchial asthma, heart volume, max-oxygen uptake lung volumes physical exercise prepubertal boys pulmonary ventilation, total hemoglobin

Many children with bronchial asthma are limited in their physical activity and are routinely exempted from physical education in school and from sports in their leisure time Parents and teachers are often overprotective and the children may become afraid of developing exercise induced asthma (EIA) Physical inactivity in adolescence can lead to a lowering of the working capacity (21) and difficulties in keeping up with healthy children This in turn may lead to social isolation, feelings of being a loser and loss of self confidence It is therefore important for both medical and psychological reasons to encourage children with asthma to take part in regular physical activity in order to overcome their fear of developing EIA, and to allow them as normal development as possible

Various techniques and conditioning programmes for asthmatic children have been described Most of them are based on calisthenics and activities for developing body strength and motor skills More strenuous training has also been used in a few studies (9, 10 20, 21) Strick (26), Fitch (9) and Oseid et al (22) have reviewed the literature In addition to psychological benefits, almost all studies report improved muscular strength and general fitness but the effects on pulmonary function and maximal oxygen uptake vary greatly

The purpose of this study is to evaluate various physical activities which enables asth

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Table 2 *Mean values and standard errors of the mean for some physiological variables, determined in asthmatic boys during long-term physical training, the training group: TG (n=11) and during corresponding observation time in the non training group: NTG (n=9)*

For explanation of abbreviations, see the text

	Initial study		After 1 year		After 2 years	
	TG	NTG	TG	NTG	TG	NTG
THb g	322 ±15 8	335 ±16 1	372 ±27 0	386 ±17 2	434 ±32 6	444 ±27 4
Hb conc, g/l	138 ±1 0	137 ±3 1	136 ±2 0	137 ±2.7	140 ±4 3	141 ±5 4
HV, ml	378 ±17 8	402 ±28 5	440 ±28 1	453 ±38 5	462 ±37 3	520 ±40 0
W_{170} w	64 2±4 7	72 4±6 5	85 5±5 1	88 1±9 2	96 1±5 4	93 7±13 1
V_{O_2} max l/min	1 59±0 07	1 74±0 11	1 89±0 08	1 97±0 16	2 05±0 11	2 13±0 24
V_E max, l/min	59 6±3 0	64 4±3 7	70 7±3 8	69 7±5 1	79 6±4 6	70 5±8 0
HR max beats/min	197 ±3 5	198 ±3 2	193 ±3 0	196 ±2 8	195 ±3 3	201 ±2 8

in fingertip blood (14) Duplicate determinations of spirometric variables, Hb, THb and BV, were made in the initial study (2) No significant differences were found between the two determinations Heart volume (HV) was determined in the prone position with bi plane radiograms (17)

During the winter training camp, dynamic spirometric determinations were made using a Vitalograf spirometer Exercise was performed on an electrically braked bicycle ergometer (Elema) at a pedalling rate of 60 rpm Oxygen uptake (V_{O_2}) was determined by collecting expired air in Douglas bags and the volumes were measured in a balanced spirometer Analysis of expired gas fractions O_2 and CO_2 was made using the micro-Scholander technique (23)

Heart rate was measured on ECG tracings, and the respiratory rate was calculated by auscultation for at least 30 sec while collecting expired air for gas analysis Blood gas analysis was made from arterialised capillary blood samples using a blood gas analyser (Instrument Laboratory model 113) In the second investigation after training for one year arterial blood samples were obtained for analysis The blood lactate concentration was determined on arterialised capillary blood using a colorimetric method (1)

Procedure

Physical examinations were performed, pertinent clinical data were recorded and lung function, blood volume, heart volume oxygen uptake and working capacity were studied in all twenty boys on three different occasions with an interval of one year between the investigations The same methods and records were used in all studies The laboratory procedure is earlier described in detail (2, 12 13)

[illegible]

late the mean S D and S E M Differences between the TG and NTG were assessed by the unpaired *t* test whereas changes within groups on different investigations were analysed by comparing paired intra individual observa-

Training programme

Physical training was performed in an ordinary indoor gymnasium for one hour twice a week under the supervision of an experienced physical educational teacher over a period of 20 months The training programme was designed to avoid triggering of EIA (Table I) Thus, during the "warming up" period, no running was allowed During circuit training, the boys worked hard for short periods at each station The results were noted and compared with previous results by the same boys The training intensity was increased during the training period The children's physician was present during the introductory sessions

After five months of conditioning training, the boys went away for one week to a winter-camp in the mountains in the northern part of Sweden (altitude 600 m) under the medical supervision of two of us The purpose of this trip was to see how much strenuous cross country skiing these asthmatic boys could perform at low temperatures A 2000 m ski race was clocked every day Each boy tried to improve his own record rather than compete with the other boys The total amount of ski training for each boy was 22000 m during the winter camp No drugs were given before the training sessions

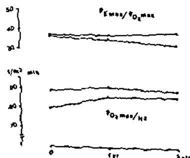


Fig 2 Values for the ventilatory coefficient (V_E max/ V_{O_2} max) and oxygen uptake, corrected for the influence of growth (V_{O_2} max/H³) at maximal exercise in 20 asthmatic boys during long term physical training Symbols as in Fig 1

Table 1. *Physical training programme used for eleven boys with bronchial asthma in an indoor gymnasium*

1.	10 min	Warming up period—no running
2.	5 min	Respiratory exercises
3.	20 min	Circuit training at five stations - skipping rope - travelling on the horizontal bar - sit-ups at gymnasium ribs - body-lifting movements - step test on bench
4.	5 min	Training on bar and rope
5	20 min	Team games basket ball, soccer, etc.

matic children to participate in physical education at school and in sports outside school. The effects of long-term physical training on the development and function of various cardio-respiratory variables have also been analysed.

MATERIAL

Eleven boys with bronchial asthma participated in a physical training programme for 20 months These boys formed the training group (TG) An additional nine asthmatic boys formed the non-training group (NTG) All the subjects have been described elsewhere with respect to their cardio-respiratory function and aerobic capacity (2, 12, 13)

The boys in the training group (TG) were selected because they lived near the indoor gymnasium that was used for physical training. The mean age at the start of the investigation was 11 2 years, range 9 2–13 6 years Based on the classification described by Kraepelien et al (19) seven boys had severe asthma (class III) with more than ten asthma attacks/year, three had moderate asthma (class II) with five to ten attacks/year, and one had mild asthma (class I) with less than five attacks/year All had a long history of perennial breathing disturbances The mean age at the onset of asthma was 2 2 years Four boys were on long term hormone therapy Prior to the study, all boys had a careful allergological work-up with skin tests and bronchial challenge Six were positive and hyposensitization was performed in these cases Before the study started only three boys participated regularly and two boys occasionally in physical education at school Six boys took part in some form of sport in their leisure time Three boys were physically inactive both at school and during their leisure time

The mean age of the boys in *the non training group* (NTG) was 11.2 years, range 9 2–13 0 years Using Kraepelien and coworkers' classification, two boys had mild (class I), four had moderate (class II), and three severe asthma (class III) The mean age at onset of symptoms was 1 6 years Six boys had a positive allergological work-up and hyposensitization was performed in these cases Two were on long term hormone treatment Five boys participated regularly and two occasionally in physical education at school Four took part in some form of sport in their leisure time One was physically inactive both at school and during his leisure time

In all but two of the asthmatic boys body height and weight were within normal limits for healthy Swedish boys (16) Both of these boys were below 2 S D for height because of steroid treatment No significant differences were found between the TG and NTG in this respect During the observation period the development of height and weight continued as expected. Before the start of the training period, there were only minor differences between the TG a [illegible] and circulatory [illegible] power In the TG, [illegible] cases, lung volumes were somewhat larger than in the NTG Forced expiratory flow and its percentage of the vital capacity were slightly decreased which indicated the presence of bronchial obstruction Maximal aerobic power was somewhat increased in the NTG, but the differences were not significant, however

METHODS

Body height and weight were carefully measured on the morning prior to the laboratory investigations These values were compared with normal values for Swedish boys (16) Static lung volumes, total lung capacity (TLC) and its subdivisions were measured by the closed-circuit helium dilution technique (3), and dynamic lung function, forced vital capacity (FVC) and forced expiratory volume during the first second (FEV_1) were determined using a Bernstein spirometer Total hemoglobin (THb) was measured with the alveolar CO method (24) and blood volume (BV) was calculated from THb and hemoglobin concentration (Hb)

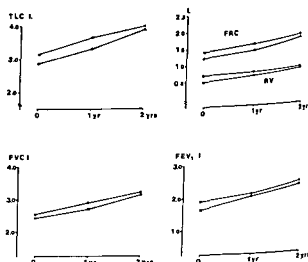


Fig 1 Mean values for some static and dynamic lung volumes in 20 asthmatic boys during the 26 months observation period Filled symbols denote the training group, TG (n=11) and open symbols the non training group, NTG (n=9)

Table 2. Mean values and standard errors of the mean for some physiological variables, determined in asthmatic boys during long-term physical training, the training group: TG (n=11) and during corresponding observation time in the non training group NTG (n=9)

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Physical examinations were performed, pertinent clinical data were recorded and lung function, blood volume,

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Before and after the one week winter training camp Bernstein spirometry and exercise testing were done.

The usual statistical methods were employed to calculate the mean, S.D. and S.E.M. Differences between the TG and NTG were assessed by the unpaired *t* test whereas changes within groups on different investigations were analysed by comparing paired intra individual observations (25).

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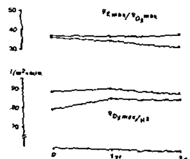


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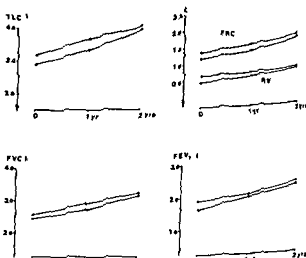
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METHODS

Body height and weight were carefully measured on the morning prior to the laboratory investigations These values were compared with normal values for Swedish boys (16) Static lung volumes, total lung capacity (TLC) and its subdivisions were measured by the closed-circuit helium [illegible] calculated from THb and hemoglobin concentration (Hb)



[illegible] TG (n=11) and open symbols the non training group, NTG (n=9)

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THb g	322 ±15 8	335 ±16 1	372 ±27 0	386 ±17 2	434 ±32 6	444 ±27 4
Hb conc g/l	138 ±1 0	137 ±3 1	136 ±2 0	137 ±2 7	140 ±4 3	141 ±5 4
HV ml	378 ±17 8	402 ±28 5	440 ±28 1	453 ±38 5	462 ±37 3	520 ±40 0
W_{m} w	64 2±4 7	72 4+6 5	85 5±5 1	88 1±9 2	96 1±3 4	93 7±13 1
V_{O_2} max l/min	1 59±0 07	1 74±0 11	1 89±0 08	1 97±0 16	2 05±0 11	2 13±0 24
V_E max l/min	59 6±3 0	64 4±3 7	70 7±3 8	69 7±5 1	79 6±4 6	70 5±8 0
HR max beats/min	197 ±3 5	198 ±3 2	193 ±3 0	196 ±2 8	195 ±3 3	201 ±2 8

in fingertip blood (14) Duplicate determinations of spirometric variables Hb THb and BV were made in the initial study (2) No significant differences were found between the two determinations Heart volume (HV) was determined in the prone position with bi plane radiograms (17)

During the winter training camp dynamic spirometric determinations were made using a Vitalograf spirometer Exercise was performed *on an electrically braked bicycle* ergometer (Elema) at a pedalling rate of 60 rpm Oxygen uptake (V_{O_2}) was determined by collecting expired air in Douglas bags and the volumes were measured in a balanced spirometer Analysis of expired gas fractions O_2 and CO_2 was made *using the micro-Scholander technique* (23)

...nd the ... at least ... Blood gas analysis was made *from arterialised* capillary blood samples using a blood gas analyser (Instrument Laboratory model 113) In the second investigation after training for one year arterial blood samples were obtained for analysis The blood lactate concentration was determined on arterialised capillary *blood* using a colorimetric method (1)

Procedure

Physical examinations were performed pertinent clinical data were *recorded and* lung function blood volume heart volume oxygen uptake and working capacity were studied in all twenty boys on three different *occasions* with an interval of one year between the investigations The same methods and records were used in all studies The laboratory *procedure* is earlier described in detail (2 12 13)

Before and after the one week winter training camp *Bernstein* spirometry and exercise testing were done

The usual statistical methods were employed to calculate the mean S D and S E M *Differences* between the TG and NTG were assessed by the unpaired t test where as changes within groups on different investigations were analysed *by* comparing paired intra individual observations (25)

Training programme

Physical training was performed in an ordinary indoor gymnasium for one hour twice a week under the supervision of an experienced physical educational teacher over a period of 20 months The training programme was designed to avoid triggering of EIA (Table 1) Thus, during the warming up period no running was allowed During circuit training the boys worked hard for short periods at each station The results were noted and compared with previous results by the same boys The training intensity *was increased* during the training period The children s physician was present during the introductory sessions

After five months of conditioning training the boys went away for one week to a winter-camp in the mountains in the northern part of Sweden (altitude 600 m) under *the medical* supervision of two of us The purpose of this trip was to see how much strenuous cross country skiing these asthmatic boys could *perform at low temperatures* A 2 000 m ski race was clocked every day Each boy tried ...

... given before the training sessions

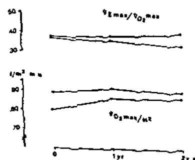


Fig 2 Values for the ventilatory coefficient (V_E max/ V_{O_2} max) and oxygen uptake corrected *for the influence* of growth (V_{O_2} max/H²) at maximal exercise in 20 asthmatic boys during long term physical training Symbols as *in Fig 1*

Table 1. *Physical training programme used for eleven boys with bronchial asthma in an indoor gymnasium*

1.	10 min	Warming-up period—no running
2.	5 min	Respiratory exercises
3.	20 min	Circuit training at five stations – skipping rope – travelling on the horizontal bar – sit-ups at gymnasium ribs – body-lifting movements – step test on bench
4	5 min	Training on bar and rope
5	20 min	Team games basket ball, soccer, etc.

matic children to participate in physical education at school and in sports outside school. The effects of long-term physical training on the development and function of various cardio-respiratory variables have also been analysed.

MATERIAL

Eleven boys with bronchial asthma participated in a physical training programme for 20 months These boys formed the training group (TG) An additional nine asthmatic boys formed the non training group (NTG) All the subjects have been described elsewhere with respect to their cardio-respiratory function and aerobic capacity (2, 12, 13)

The boys in the training group (TG) were selected because they lived near the indoor gymnasium that was used for physical training The mean age at the start of the investigation was 11 2 years, range 9 2–13 6 years Based on the classification described by Kraepelien et al (19) seven boys had severe asthma (class III) with more than ten asthma attacks/year, three had moderate asthma (class II) with five to ten attacks/year, and one had mild asthma (class I) with less than five attacks/year All had a long history of perennial breathing disturbances The mean age at the onset of asthma was 2 2 years Four boys were on long-term hormone therapy Prior to the study, all boys had a careful allergological work-up with skin tests and bronchial challenge Six were positive and hyposensitization was performed in these cases Before the study started only three boys participated regularly and two boys occasionally in physical education at school Six boys took part in some form of sport in their leisure time Three boys were physically inactive both at school and during their leisure time.

The mean age of the boys in the non training group (NTG) was 11 2 years, range 9 2–13 0 years. Using Kraepelien and coworkers' classification, two boys had mild (class I), four had moderate (class II), and three severe asthma (class III) The mean age at onset of symptoms was 1 6 years Six boys had a positive allergological work up and hyposensitization was performed in the cases Two were on long term hormone treatment Five boys participated regularly and two occasionally in physical education at school Four took part in some form of sport in their leisure time One was physically inactive both at school and during his leisure time

In all but two of the asthmatic boys body height and weight were within normal limits for healthy Swedish boys (16) Both of these boys were below 2 S D for height because of steroid treatment No significant differences were found between the TG and NTG in this respect During the observation period the development of height and weight continued as expected Before the start of the training period, there were only minor differences between the TG and the NTG with respect to respiratory and circulatory dimensions and function and to aerobic power. In the TG, which consisted of more severe asthma cases, lung volumes were somewhat larger than in the NTG. Forced expiratory flow and its percentage of the vital capacity were slightly decreased which indicated the presence of bronchial obstruction Maximal aerobic power was somewhat increased in the NTG, but the differences were not significant, however.

METHODS

Body height and weight were carefully measured on the morning prior to the laboratory investigations These values were compared with normal values for Swedish boys (16) Static lung volumes, total lung capacity (TLC) and its subdivisions were measured by the closed-circuit helium dilution technique (3), and dynamic lung function, forced vital capacity (FVC) and forced expiratory volume during the first second (FEV_1) were determined using a Bernstein spirometer Total hemoglobin (THb) was measured with the alveolar CO method (24) and blood volume (BV) was calculated from THb and hemoglobin concentration (Hb)

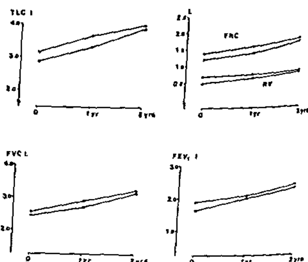


Fig 1 Mean values for some static and dynamic lung volumes in 20 asthmatic boys during the 26 months observation period Filled symbols denote the training group, TG (n=11) and open symbols the non training group,

the somewhat more severe asthma in the boys who belonged to the training group Compared with healthy children these boys showed signs of impaired lung function with increased lung volume ratios (FRC/TLC=44 4%, RV/TLC= 22 5%) and low FEV% (66 7%) (6) The maximal aerobic power (1 6 vs 1 9 l/min) was somewhat lower than in different groups of healthy boys (8) and this was true even when correction was made for body height

It was anticipated that, as in previous studies (15, 20, 21) participation in the training programme would improve aerobic work capacity but this was not confirmed The findings likewise do not concord with the opinion that physical training before and during puberty is essential for enhancement of maximal aerobic power (29 5) However, more recent studies have shown that around 12 and 13 years of age increases in circulatory dimensions parallel body growth and work capacity is less affected by training than at other ages (18 27)

The degree of training intensity that was used in this study may also explain why physical training failed to affect the variables studied The programme was designed to allow the boys to enjoy the physical education given at school and to avoid triggering EIA Before the training programme was initiated the TG had a somewhat lower working capacity at submaximal and maximal levels than the NTG (Fig 3) and a lower V_{O_2} max (Fig 2) No significant changes were noted in the TG during the long term physical training The TG increased its values more than the NTG but the increase was small and not significant

The dimensions of the pulmonary and cardiovascular systems were unaffected by physical training (Fig 3) This is not surprising as an increase in lung volumes has only been demonstrated after intensive training in swimming over a long period (7) The differences in FEV% between the TG and the NTG diminished during the observation period but FEV% varies considerably in asthmatic children and it is not possible to draw any conclusions from these results

During the one week winter camp, the training intensity was high and all boys developed EIA after the 2000 m ski race The FEV_1 was reduced by a mean of 19% approximately 4 min after exercise The reduction might have been greater if the determinations had been made 10–15 min after the race as the maximal effect of EIA on expiratory flow usually occurs after that time interval (11) In spite of the high exercise intensity, there was no increase in maximal V_{O_2} after the training camp This is probably because the one week training period was too short but there was a small increase in some ventilatory variables The total ventilation at maximal work rose from 61 9 to 68 7 l ($p<0$ 01), the FEV_1 from 1 8 to 1 9 (n s) and the maximal work from 124 to 132 W ($p<0$ 01) This suggests that it is possible to influence the voluntary ventilatory function and working capacity with unchanged maximal V_{O_2} if the training intensity is sufficiently high

A higher exercise intensity has been reported to improve pulmonary function and working capacity (9, 21) but in these studies the children inhaled disodium cromoglycate (Intal®) before the training sessions This makes it possible to attain a good training effect on pulmonary function and aerobic capacity

The findings in this study show that asthmatic boys can participate in a physical training programme similar to the physical education given at school without premedication if minor modifications are made However, the use of DSCG or sympatomimetic drugs before the training sessions can be recommended so that asthmatic children can participate to the greatest possible extent in physical activities without EIA If the aim of the physical training is to increase the working capacity and aerobic power it is necessary to premedicate the children

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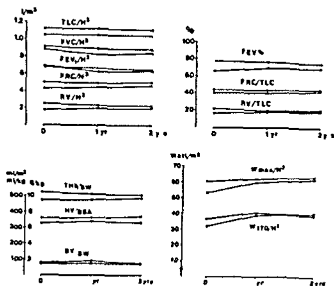


Fig 3 Mean values for respiratory dimensions and function circulatory dimensions and maximal work load corrected for influence of growth in two groups of asthmatic boys during 26 months observation time Symbols as in Fig 1

RESULTS

All boys participated in the entire training programme A mean of 9 of the 11 boys were present at each lesson Two boys (class III), were absent from training more frequently mainly because of upper respiratory tract infections and asthma Two other boys (classes II and III) developed exercise-induced asthma (EIA) more often than the others but they trained the most EIA, when present almost always subsided within 5–10 min without medical treatment Inhalation of β_2-receptor stimulators was only required occasionally Team games, such as basketball and soccer, were the commonest causes of EIA All boys were very satisfied with the training and no negative effects were noted

During the observation period, all values for respiratory (Fig 1) and circulatory dimensions increased in the TG and NTG (Table 2) During maximal exercise, cardio respiratory function also increased and both groups showed good adaptation to heavy physical work In the TG, total ventilation (V_E) rose from 59 6 to 79 6 l/min (33 6%) whereas in the NTG it rose from 64 4 to 70 5 l/min (9 5%) The maximal oxygen uptake (V_{O_2} max) was initially somewhat lower in the TG, 1 59 l/min, than in the NTG, 1 74 l/min, but it increased during the observation period by 28 9 and 22 4% respectively. Thus, the ventilatory coefficient (V_E max/V_{O_2} max) increased in the TG from 37 5 to 38 8 ($p<0$ 05), Fig 2 The maximal work load rose 38 7% in the TG and 28 6% in the NTG As the boys were still growing, all values had to be corrected for height (4) After correction none of these differences were significant Thus the TG did not differ from the NTG in any respect and long term physical training had no apparent influence on respiratory and circulatory dimensions and function in these boys (Fig 3)

During the one-week winter training camp there was a small increase in the total ventilation (V_E max) ($p<0$ 01) and in the maximal work load ($p<0$ 01) but the maximal oxygen uptake was unchanged (Table 3)

Dynamic spirometric tests performed before and 4–6 min after the daily ski race showed lowering of the FEV_1 after exercise in all boys Hence EIA was present The lowering was most pronounced on the third and fourth days of training 23% vs 22%, and the mean 19% for the whole week The more severe asthma cases showed the most marked reduction in expiratory flow after exercise

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The small differences found for various variables at the start of the study comparing the boys of the two groups, may be attributed to

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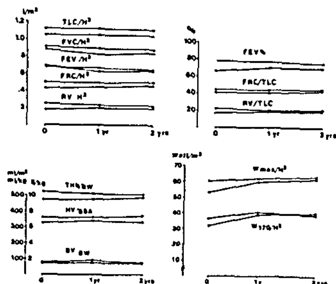


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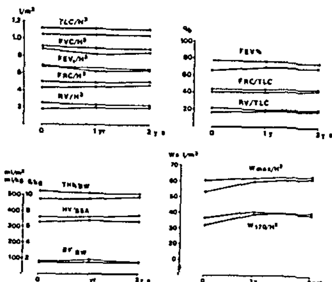


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Acta Pædiatr Scand 69 353–358, 1980

THE ROLE OF NUTRITIONAL STATUS, AIRWAY OBSTRUCTION, HYPOXIA, AND ABNORMALITIES IN SERUM LIPID COMPOSITION IN LIMITING EXERCISE TOLERANCE IN CHILDREN WITH CYSTIC FIBROSIS

A L COATES P BOYCE D MULLER M MEARNS and S GODFREY

From the Department of Paediatrics of the Hammersmith Hospital and the Queen Elizabeth Hospital for Children and the Institute of Child Health London England

ABSTRACT Coates, A L , Boyce, P , Muller, D , Mearns, M B and Godfrey, S (Department of Paediatrics, Hammersmith Hospital, Queen Elizabeth Hospital, and the Institute of Child Health, London, England) The role of nutritional status, airway obstruction, hypoxia, and abnormalities in serum lipid composition in limiting exercise tolerance in children with cystic fibrosis Acta Paediatr Scand, 69 353, 1980.—Previous work has shown that impaired exercise tolerance in children with cystic fibrosis (C F) is related to the severity of airway obstruction without elucidating the possible roles of hypoxia or malnutrition It has been suggested that poor nutrition leads to abnormalities in serum fatty acids composition, which may lead to tissue hypoxia We investigated the roles of hypoxia, pulmonary mechanics, nutritional status, and serum fatty acid composition in limiting exercise tolerance in C F In 20 children with C F , exercise tolerance, while breathing air and while breathing oxygen, was evaluated on a cycle ergometer and compared to pulmonary function tests, anthropometric data, serum lipid composition, and clinical condition The mean percent work expected from height (W_{max}) was 75, and was unchanged by O_2 W_{max} correlated significantly with the degree of respiratory impairment, the discrepancy between height and weight, and the clinical score but not serum fatty acid composition Where measured, no child at any time had an elevated end tidal $CO_2(P_{etCO_2})$ We conclude that nutritional status and airway obstruction are closely correlated with exercise tolerance in C F and that, unlike the case in adults with chronic obstructive pulmonary disease, exercise-limiting dyspnea occurs in the presence of a normal P_{etCO_2}

KEY WORDS Cystic fibrosis malnutrition, serum fatty acid composition, exercise tolerance, pulmonary mechanics

Although cystic fibrosis (C F) is a multisystem disease, most of the morbidity is usually attributed to lung disease leading to impaired pulmonary function and to digestive disorders leading to malnutrition and poor growth This has led the assessment of overall severity to be based on these two systems (25, 27) Attention has been drawn to a possible relationship between abnormal fatty acid composition in the serum tissue hypoxia, and poor clinical condition in children with C F (1, 6) In 1971, two of us demonstrated that children with C F have impaired exercise tolerance, which is correlated with abnormalities of lung function and poor clinical condition, and suggested that impairment of pulmonary mechanics, rather than the accompanying hypoxemia, was responsible for the exercise limitation (9) A recent study (15), using a respiratory muscle training program, has demonstrated an improvement in respiratory muscle performance, raising the possibility that respiratory muscle weakness may limit exercise tolerance Davies et al (5) have shown that maximum oxygen uptake on a cycle ergometer is best correlated with lean leg mass, in other words, leg

Dr Coates was a Fellow of the Canadian Medical Research Council Miss Boyce was supported by the British Cystic Fibrosis Foundation

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Submitted Dec 14 1978
Accepted Nov 2 1979

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Acta Pædiatr Scand 69 353–358, 1980

THE ROLE OF NUTRITIONAL STATUS, AIRWAY OBSTRUCTION, HYPOXIA, AND ABNORMALITIES IN SERUM LIPID COMPOSITION IN LIMITING EXERCISE TOLERANCE IN CHILDREN WITH CYSTIC FIBROSIS

A L COATES P BOYCE D MULLER, M MEARNS and S GODFREY

From the

ABSTRACT Coates, A L , Boyce, P , Muller, D , Mearns, M B and Godfrey, S (Department of Paediatrics, Hammersmith Hospital Queen Elizabeth Hospital, and the Institute of Child Health, London, Englan
tion, hypoxia, and abnormalities in seru
in children with cystic fibrosis Acta Pa
shown that impaired exercise tolerance in children ... to the severity of airway obstruction without elucidating the possible roles of hypoxia or malnutrition It has been suggested that poor nutrition leads to abnormalities in serum fatty acids composition, which may lead to tissue hypoxia We investigated the roles of hypoxia, pulmonary mechanics, nutritional status, and serum fatty acid composition in limiting exercise tolerance in C F In 20 children with C F , exercise tolerance, while breathing air and while breathing oxygen, was evaluated on a cycle ergometer and compared to pulmonary function tests, anthropometric data, serum lipid composition, and clinical condition The mean percent work expected from height (W_{max}) was 75, and was unchanged by O_2. W_{max} correlated significantly with the degree of respiratory impairment, the discrepancy between height and weight, and the clinical score but not serum fatty acid composition Where measured, no child at any time had an elevated end tidal CO_2(P_{ETCO_2}) We conclude that nutritional status and airway obstruction are closely correlated with exercise tolerance in C F and that, unlike the case in adults with chronic obstructive pulmonary disease, exercise-limiting dyspnea occurs in the presence of a normal P_{ETCO_2}

KEY WORDS *Cystic fibrosis*, malnutrition, serum fatty acid composition, exercise tolerance, pulmonary mechanics

Although cystic fibrosis (*C F*) is a multisystem disease, *most of the* morbidity is usually attributed to lung disease leading to impaired pulmonary function and to digestive disorders leading to malnutrition and poor growth This has led the assessment of overall severity to be based on these two systems (25, 27) Attention has been drawn to a possible relationship between abnormal fatty acid composition in the serum, tissue hypoxia, and poor clinical condition in children with C F (1, 6) In 1971, two of us demonstrated that children with C F have impaired exercise tolerance, which is correlated with abnormalities of lung function and poor clinical condition, and suggested that impairment of pulmonary mechanics, rather than the accompanying hypoxemia, was responsible for the exercise limitation (9) A recent study (15), using a respiratory muscle training program, has demonstrated an *improvement* in respiratory muscle performance, raising the possibility that respiratory muscle weakness may limit exercise tolerance Davies et al (5) have shown that maximum oxygen uptake on a cycle ergometer is best correlated with lean leg mass, in other words, leg

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KEY WORDS Cystic fibrosis, malnutrition, serum fatty acid composition, exercise tolerance, pulmonary mechanics

Although cystic fibrosis (C F) is a multisystem disease, most of the morbidity is usually attributed to lung disease leading to impaired pulmonary function and to digestive disorders leading to malnutrition and poor growth This has led the assessment of overall severity to be based on these two systems (25, 27) Attention has been drawn to a possible relationship between abnormal fatty acid composition in the serum tissue hypoxia, and poor clinical condition in children with C F (1, 6) In 1971, two of us demonstrated that children with C F have impaired exercise tolerance, which is correlated with abnormalities of lung function and poor clinical condition, and suggested that impairment of pulmonary mechanics, rather than the accompanying hypoxemia, was responsible for the exercise limitation (9) A recent study (15), using a respiratory muscle training program, has demonstrated an improvement in respiratory muscle performance, raising the possibility that respiratory muscle weakness may limit exercise tolerance Davies et al (5) have shown that maximum oxygen uptake on a cycle ergometer is best correlated with lean leg mass, in other words, leg

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max ats/ n)	% Linoleic acid	% Oleic acid
6	35	31
4	25	30
0	32	32
96	40	23
4	24	35
72	33	34
6	48	28
64	32	35
84	38	31
68	–	–
60	28	35
84	42	28
72	–	–
76	–	–
194	39	24
160	27	33
184	38	33
184	32	30
180	42	28
168	27	32

ᵃ Radial artery sample

breathing room air and once while breathing pure humidified O_2. The order in which these were administered was assigned randomly. At least a 90-min rest period was allowed between each run. During the test inspired minute ventilation and heart rate were recorded. The final work load completed was compared to that expected for both the child's height and lean leg volume. In five children the exercise test was repeated with expiratory gas being continually sampled and analyzed for end tidal CO_2 (P_{ETCO_2}) on a mass spectrometer.

The fatty acid composition of the cholesterol ester fraction of the serum was measured by gas chromatography (7). The children were not fasting at the time of the venous blood sample and were on a normal diet with enzymes added to control stool abnormalities. The analysis of the cholesterol ester fraction rather than total serum fatty acid composition was done to avoid fluctuations due to variations in dietary intake (21).

RESULTS

The age, sex, anthropometric data and Shwachman scores are listed in Table 1. Only six of the twenty children were at or above the fiftieth percentile for height and only three

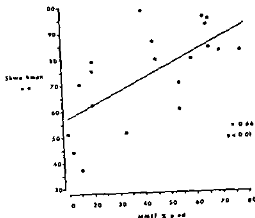


Fig. 1. The Shwachman score plotted against the maximum midexpiratory flow rate (MMEF) as a percent predicted from height.

were above it for weight. This discrepancy between height and weight is quantitated by the BMP, the mean value of which was 90. Shwachman scores for the children varied from 37 to 98. Five were considered excellent (86–100), 7 good (71–85), 4 mild (56–70), 3 moderate (41–55), and 1 severe (<40) (25). With increasing severity of disease there was an increasing discrepancy betweeen the height and weight percentiles. This is illustrated by the correlation between the Shwachman score and the BMP ($r=0.55$, $p<0.02$).

The tests for airway obstruction, namely the MMEF (Table 1) and MVV correlated well with each other ($r=0.8$, $p<0.001$) and since the MMEF is a simpler test to do and more widely used, it was used in all comparisons as a percent predicted from height. Fig. 1 shows the correlation between the Shwachman score and the MMEF, and Fig. 2 shows the correlation between the MMEF and the BMP. Hence, there is a correlation of both clinical score and nutritional state with the degree of airway obstruction. The parameters of gas exchange and the (A-a)O_2 gradient correlated poorly with the MMEF ($p<0.1$) and not with the Shwachman score (Table 1).

Six of the children accomplished more work while breathing O_2 compared to breathing air, and two accomplished less work, with the ex-

Table 1. *The patient, age, sex, the anthropometric data, the body mass percentile (BMP) and Shwachman score, MMEF as a percent predicted from height, the alveolar arterial gradient (A-a)O₂, W_{max} as a percent predicted from height, the W_{max} as a percent predicted from lean leg volume, the maximum heart rate in air, and the percent oleic and linoleic acid esters in cholesterol ester fraction of the patients*

Pats	Age (years)	Height (cm)	Weight (kg)	BMP	Swachman score	MMEF (% pred)	(A a)O_2 (kPa)	W_{max} (% pred height)	W_{max} (% pred leg vol)
Males									
JM	16	160	56 5	88	75	20	6 3*	53	63
GA	15	153	44 0	98	86	44	2 7	103	102
AN	15	143	33 3	85	82	70	4 1*	85	106
MG	14	178	60 9	107	82	78	4 0*	97	100
BH	14	140	27 9	79	70	10	4 0	55	61
PH	13	140	30 5	91	60	54	3 1	82	84
BS	12	141	31 2	94	79	59	3 1	91	82
HS	12	135	28 5	93	51	10	4 9*	61	53
KB	11	131	25 2	90	94	66	3 1	100	97
CB	11	139	34 5	108	95	64	3 6	91	88
JH	11	135	24 8	83	44	12	5 1	50	60
KH	10	132	25 1	91	62	20	4 7*	57	54
DH	9	126	22 4	90	79	45	3 2	100	90
ZK	8	129	25 5	99	92	65	–	82	82
Females									
RP	15	161	37 5	74	51	33	3 5	46	54
SH	13	144	32 7	88	70	54	4 8*	86	78
SA	12	139	27 5	84	79	20	3 6	78	66
BD	12	149	32 5	86	98	40	–	76	70
WK	12	135	23 5	76	37	15	–	49	51
JC	9	126	21 6	86	83	66	–	86	61

muscle and bone They further demonstrated that this relationship is unaltered by malnutrition, which reduces both work and lean leg mass, and suggested that lean leg volume rather than the usual index of height was a better predictor of expected work capacity in children who suffer from malnutrition (4) The purpose of this report is to assess what relationship, if any, exists between height and body proportions, pulmonary mechanics, gas exchange, clinical scoring, serum fatty acid composition, and exercise tolerance while breathing air and while breathing oxygen in children with C F

MATERIALS AND METHODS

In tw
clinic
(25), ...
knowledge of the other measurements obtained Height

and weight were measured and percentiles were determined for each child (26) Total leg volume and lean leg volume (LV) were calculated using the method of Jones & Pearson (14) The body mass index (16 22) (weight/height²) was calculated in order to have an assessment of weight in relation to height This was adjusted to account for the normal changes in body proportions with age by dividing the body mass index for each child by the index calculated for a child of the same age but at the fiftieth percentile for both height and weight (26) and then multiplied by 100 to give the body mass percentile (BMP)

Maximum mid expiratory flow rates (MMEF) and vital capacity (VC) were measured by water spirometry (23) Maximum voluntary ventilation (MVV) was determined (3) Resting arterialized earlobe blood was drawn (10) on 10 of the children and in 6 other children radial artery blood was drawn $P_{a_{O_2}}$ $P_{a_{CO_2}}$ and pH were determined and the alveolar arterial oxygen gradient

child exercised continuously until exhaustion the work load on the ergometer being increased by a fixed increment each minute The increases in the load (5 10 or 15 watts) depended on the size and condition of the child and were chosen so that exhaustion would occur within 5 to 10 min This test was performed twice once while

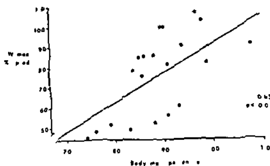


Fig 5 The W_{max} as a percent predicted from height plotted against the body mass percentile

tion between the percentage oleic or linoleic acids in cholesterol esters and the Shwachman score, the W_{max} as a percent predicted from either height or lean leg volume, the MMEF, the (A a)O_2, or the BMP

DISCUSSION

This study has demonstrated that the combined effects of airway obstruction and malnutrition play a major role in the limitation of exercise tolerance in children with C F The poor performance of those children whose BMP was less than 85 (Fig 5) leads us to speculate that malnutrition leads first to a loss of body fat and then to muscle wasting While the loss of muscle in the legs would have a direct effect on performance on a cycle ergometer similar wasting of the respiratory muscles could also influence respiratory function, a suggestion compatible with the data of Keens et al (15) This relative thinness has recently been emphasized by Kraemer et al (17) who showed that it is related to both poor survival and the extent of pulmonary disease

The mean heart rate at the final work load accomplished in air (fH_{max}) was 175 which is lower than the 195 found in normal children (8) There was a tendency for the children with the lower MMEF's and Shwachman scores to have lower fH_{max} s In 8 children in whom the test was repeated to evaluate the variation in the results, a W_{max} within one work load of the previous W_{max} and a very similar heart rate were obtained, suggesting that willingness to cooperate was not a factor When the children were asked what made them stop pedalling they all cited dyspnea and fatigue in their legs Since there is no reason to believe that children with C F cannot increase their heart rate in response to stress, the role that the maximum heart rate may play in limiting exercise in normal children (8) does not appear to be a major factor in children with C F

The data suggest that breathing oxygen, as compared to breathing air, leads to a slight increase in W_{max}, and a higher P_{etCO_2} with a lower ventilation at the same work load This has been described previously in normal adults (19, 28) Hence, in our subjects, hypoxemia did not appear to be the major limiting factor of exercise tolerance

The striking correlation between the MMEF and W_{max} (Table 1, Fig 3) strongly suggests that airway obstruction plays a major role in the limitation of exercise in our patients What is more difficult to explain is why this should be so In the children in whom P_{etCO_2} at W_{max} was measured, the values were always within the normal range While there is a tendency for the P_{etCO_2} to be lower than the P_{aCO_2} in patients with airway obstruction, the difference would be less than 0 67 kPa (13) However, the trend for the children with the lowest MMEF's to use a larger portion of their MVV, a finding that has also been previously reported (9), leaves us with the postulate that exercise-limiting dyspnea occurs as ventilation approaches the MVV but before the onset of respiratory failure This is unlike the case in adult patients with chronic bronchitis (12), who tend to have an elevated P_{aCO_2} on exercise

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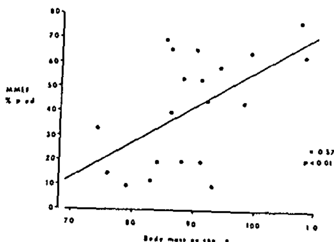


Fig 2 The maximum mid expiratory flow rate (MMEF) as a percent predicted from height plotted against the body mass percentile

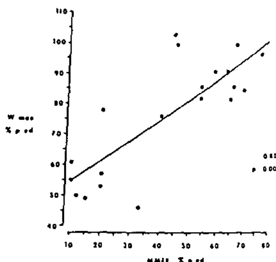


Fig 3 The W_{max} as a percent predicted from height plotted against the MMEF as a percent predicted from height

ception of two children, the difference was never more than one work load The mean maximum heart rate while breathing air (±S D) was 175±10 and while breathing oxygen at the same work load was a mean of six beats less, these differences were significant ($p<0$ 01, Student's paired *t*-test) The ventilation while breathing O_2 was 12% less than when breathing air ($p<0$ 001), and there was a tendency for children whose MMEF was less than 35% predicted to use an increasingly larger fraction of the MVV at each successive work load than those with a higher MMEF, who always had some respiratory reserve at the final work load The five children whose clinical score ranged from 37 to 83 and whose P_{etCO_2} was measured at the last work load completed, had slightly higher P_{etCO_2} values while breathing O_2, a mean of 5 1 kPa with a range of 4 9–5 3 compared to 4 6 with a range of 4 1–5 2 while breathing air

There was no difference between the maximum work accomplished as predicted from height, a mean of 76% compared to that predicted from lean leg volume, a mean of 75% for the group as a whole (paired *t* test) In a multiple regression analysis of both MMEF and BMP against W_{max}, the *r*-value is 0 81 with $p<0$ 0001, suggesting a combined effect of both airway obstruction and malnutrition on exercise tolerance The partial correlations, 0 68 for MMEF and 0 40 for BMP, indicate that airway obstruction is the more important of the two factors The W_{max} expressed as a percent predicted from height correlates with the MMEF, the Shwachman score and BMP (Figs 3, 4 and 5)

The percentages of linoleic acid and oleic acid in the cholesterol ester fraction are shown in Table 1 In 10 of the 17 patients studied the percentage of linoleic acid fell outside the normal range (i e <37%, mean −2 S D) and of the remainder, 6 had levels less than 43% (mean −1 S D) *The percent oleic acid was* increased in all but 2 of the patients (i e >27% mean +2 S D) There was no correla

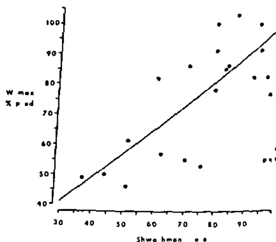


Fig 4 The W_{max} as a percent predicted from height plotted against the Shwachman score

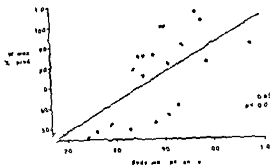


Fig 5 The W_{max} as a percent predicted from height plotted against the body mass percentile

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DISCUSSION

This study has demonstrated that the combined effects of airway obstruction and malnutrition play a major role in the limitation of exercise tolerance in children with C F The poor performance of those children whose BMP was less than 85 (Fig 5) leads us to speculate that malnutrition leads first to a loss of body fat and then to muscle wasting While the loss of muscle in the legs would have a direct effect on performance on a cycle ergometer similar wasting of the respiratory muscles could also influence respiratory function, a suggestion compatible with the data of Keens et al (15) This relative thinness has recently been emphasized by Kraemer et al (17) who showed that it is related to both poor survival and the extent of pulmonary disease

The mean heart rate at the final work load accomplished in air (fH_{max}) was 175 which is lower than the 195 found in normal children (8) There was a tendency for the children with the lower MMEF's and Shwachman scores to have lower fH_{max}'s In 8 children in whom the test was repeated to evaluate the variation in the results, a W_{max} within one work load of the previous W_{max} and a very similar heart rate were obtained, suggesting that willingness to cooperate was not a factor When the children were asked what made them stop pedalling they all cited dyspnea and fatigue in their legs Since there is no reason to believe that children with C F cannot increase their heart rate in response to stress, the role that the maximum heart rate may play in limiting exercise in normal children (8) does not appear to be a major factor in children with C F

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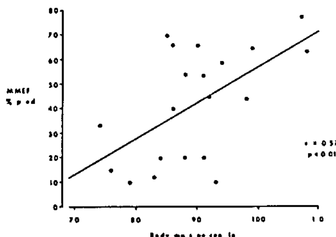


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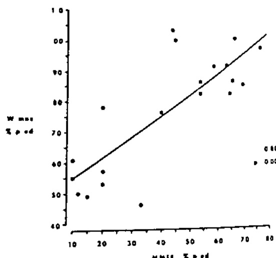


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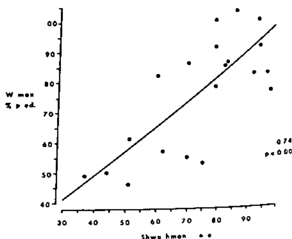


Fig 4 The W_{max} as a percent predicted from height plotted against the Shwachman score

Acta Pædiatr Scand 69 359–364, 1980

CHESTWALL COMPLIANCE IN FULL-TERM AND PREMATURE INFANTS

TILO GERHARDT and EDUARDO BANCALARI

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ABSTRACT Gerhardt, T and Bancalari, E (Department of Pediatrics, University of Miami, Florida, U S.A) Chestwall compliance in full term and premature infants Acta Pediatr Scand, 69·359, 1980.—Chestwall compliance was determined in 26 premature infants (BW 1 320±410 g, gest age 32 weeks) and in 10 full-term infants (BW 3 155±810 g) who were ventilated mechanically Chestwall compliance in premature infants was 6 4 ml/(cmH_2O×kg), decreasing with advancing gestational age to 4 2 ml/(cmH_2O×kg) in full term infants There was a linear correlation ($r=0\ 95$ and 0 79 respectively) between tidal volume and the pressure transmitted to the esophagus throughout the tidal volume range The portion of airway pressure transmitted to the esophagus depended on the infant s lung compliance Only 5% was transmitted in infants with hyaline membrane disease, 12% in newborns with

[illegible]

which could interfere with central venous return and cardiac output However, using high inspiratory pressures and continuous distending airway pressure in the absence of lung pathology may result in a decreased cardiac output The highly compliant chestwall of the premature infant may exert insufficient outward recoil and might be one of the causes of a low functional residual capacity and chronic pulmonary failure in the premature infant.

KEY WORDS Chestwall compliance, newborn infants, airway pressure, esophageal pressure transmission, lung compliance

Since the advent of neonatal intensive care, mechanical ventilation of newborns with respiratory failure has become a common practice The positive pressure required to expand the lungs must overcome pulmonary resistance plus the elastic and surface forces of the lung In addition to that, part of the pressure is used to move the chestwall Sufficient information is now available regarding airway resistance and lung compliance in newborns, but little is known about chestwall compliance in full term and premature infants

The decrease in central venous return and pulmonary blood flow, which is described as a detrimental effect of positive pressure ventilation in the adult, is related to an increase in intrapleural pressure This increase in pleural pressure depends, among other factors, on the compliance of the chestwall

The elasticity of the thorax plays an important role in determining the resting volume of the lungs A very compliant chestwall may not maintain an adequate transpulmonary pressure at end expiration, resulting in a decreased functional residual capacity, alveolar collapse and hypoxemia

Reliable data on chestwall compliance in newborns will, therefore, be helpful in 1) the management of mechanically ventilated newborns, 2) explaining some of the complications of this mode of therapy, and 3) contributing to a better understanding of the mechanics of breathing in newborns

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Abbreviations used

Paw=airway pressure P_L=transpulmonary pressure *Ppl*=intrapleural pressure, *Pes*,=esophageal pressure, V_T=tidal volume C_L=lung compliance, *Cw*=chestwall compliance *PEEP*=positive end expiratory pressure

port the recent observations of Hubbard et al (11), who found that abnormalities in blood lipids correlated with the degree of pancreatic insufficiency and not lung function

From these data we conclude that nutritional status and airway obstruction are closely correlated with exercise tolerance in C F and that, unlike the case in adults with chronic bronchitis, exercise limiting dyspnea occurs in the presence of a normal P_{aCO_2} Furthermore, in view of the close relationship with W_{max} and the clinical score, we are suggesting that exercise testing yields a simple and reproducible index of overall health in cystic fibrosis This index is less likely to be influenced by inter observer error than is a more subjective clinical evaluation

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Submitted July 5 1979
Accepted Nov 5 1979

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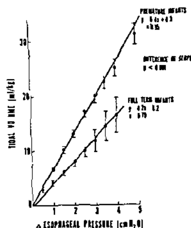


Fig 2 Relation between tidal volume and esophageal pressure in premature and full term infants (Mean ± S E of the tidal volumes are shown to avoid overlapping)

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Airway pressure was measured directly in the endotracheal tube using a Statham PM5 Pressure Transducer[3] and a Gould Transducer Coupler [4]

A feeding tube (size 5 French for the premature infants size 8 French for the full term newborns) was positioned in the mid-esophagus to record esophageal pressure changes which were measured with a Statham P23AA Pressure Transducer[3] and a third Gould Transducer Coupler [4] The system was filled with bacteriostatic water for injection from an attached syringe No formation of gas bubbles was observed by warming the system to 60°C for 2 hrs The feeding tube was flushed during recording repeatedly to clear its tip from the accumulation of mucus and saliva The volume displacement of the transducer was 0 006 mm³/cmH_2O i e low enough to transmit esophageal pressure changes with accuracy (11) The frequency response of the pressure transmitting system was tested to be linear up to 240 cycles/min

To obtain a quasi static determination of chestwall compliance inspiration was prolonged to 1 0 sec so that the tracings of airway pressure and tidal volume reached a plateau indicating that no more volume changes occurred (Fig 1) Throughout the determination the infant s ventilation was controlled by slight hyperventilation until no spontaneous breathing occurred Leaks in the system or around the endotracheal tube were easily detected as the inspiratory volume became larger than the expiratory volume Small leaks were corrected by applying slight pressure over the trachea The recordings of flow tidal volume airway pressure and esophageal pressure were made on Brush 260 Recorder [4]

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RESULTS

Results are given in Table 1 and Fig 2 In both premature and in full term infants there is a linear increase in esophageal pressure change with increasing tidal volume (r=0 95 and 0 79 respectively) The slope of the curves is significantly flatter in the full term infants, indicating that their chestwall compliance is lower Mean chestwall compliance in the premature infants was 6 4, while in the full-term infants it was 4 2 ml/(cmH_2O×kg) (p<0 001)

Chestwall compliance was similar in premature infants with patent ductus arteriosus and hyaline membrane disease The decrease in

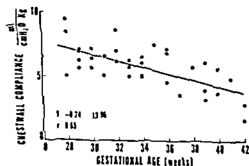


Fig 3 Relation between gestational age and chestwall compliance in 36 newborn infants

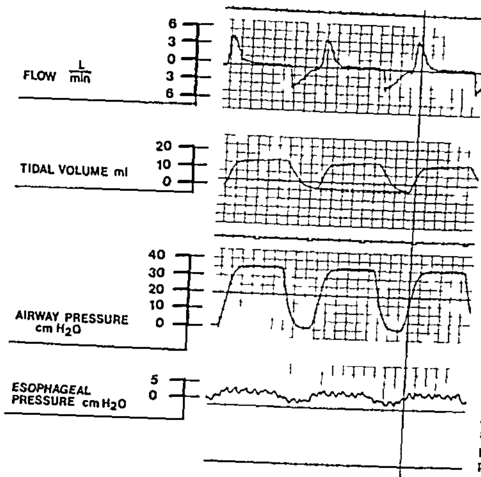


Fig 1 Typical tracing of flow t d al volume airway and esophageal pressure showing an insp ratory plateau

MATERIAL AND METHOD

Under static conditions (no flow through the airways and absence of volume changes) the airway pressure (Paw) applied to inflate the lungs is only partly used to overcome the elastic and surface forces of the lung (P_L) The other part is necessary to move the chestwall from one thoracic volume to another (ΔV) and is reflected in changes of in trapleural pressure (ΔPpl)

These volume changes occurring per intrapleural pres sure change reflect chestwall compliance (Cw)

$$Cw = \frac{\Delta V}{\Delta P pl}$$

Intrapleural pressure changes can be determined fairly accurately by measuring esophageal pressure changes (ΔPes) (4 6 12)

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Therefore pressure volume curves of the chestwall can be constructed by measuring Pes at different inspiratory volumes

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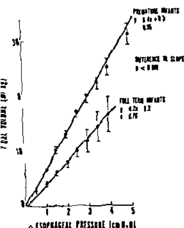


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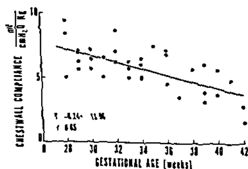


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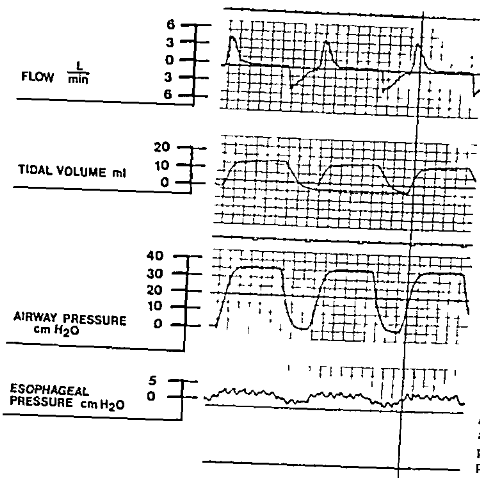


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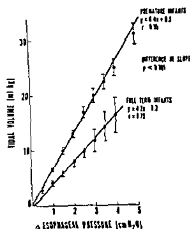


Fig 2 Relation between tidal volume and esophageal pressure in premature and full term infants (Mean ± S E of the tidal volumes are shown to avoid overlapping)

ume was obtained by electrical integration of this flow signal using a *Gould Integrator Coupler* [4] The system was calibrated with known volumes of gas using a glass syringe, and flow rates similar to those generated by the ventilator The system was tested to give accurate volume readings at positive end expiratory pressure levels of up to 20 cmH_2O and peak inspiratory pressures up to 60 cmH_2O

Airway pressure was measured directly in the endotracheal tube using a Statham PM5 Pressure Transducer[3] and a Gould Transducer Coupler [4]

A feeding tube (size 5 French for the premature infants, size 8 French for the full term newborns) was positioned in the mid-esophagus to record esophageal pressure changes which were measured with a Statham P23AA Pressure Transducer[3] and a third Gould Transducer Coupler [4] The system was filled with bacteriostatic water for injection from an attached syringe No formation of gas bubbles was observed by warming the system to 60°C for 2 hrs The feeding tube was flushed during recording repeatedly to clear its tip from the accumulation of mucus and saliva The volume displacement of the transducer was 0 006 mm^3/cmH_2O i e low enough to transmit esophageal pressure changes with accuracy (11) The frequency response of the pressure transmitting system was tested to be linear up to 240 cycles/min

To obtain a quasi static determination of chestwall compliance inspiration was prolonged to 1 0 sec, so that the tracings of airway pressure and tidal volume reached a plateau indicating that no more volume changes occurred (Fig 1) Throughout the determination the infant s ventilation was controlled by slight hyperventilation until no spontaneous breathing occurred Leaks in the system or around the endotracheal tube were easily detected as the inspiratory volume became larger than the expiratory volume Small leaks were corrected by applying slight pressure over the trachea The recordings of flow, tidal volume airway pressure and esophageal pressure were made on Brush 260 Recorder [4]

The transmission of esophageal pressure changes was frequently distorted by cardiac activity Therefore, the accuracy of pressure recordings was reduced to ±0 25 cmH_2O Because of this limitation the values of esophageal pressure changes were arbitrarily placed into 10 groups starting at 0 5 cmH_2O and rising by 0 5 cmH_2O increments to 5 0 cmH_2O The pressure limits for each group were ±0 25 cmH_2O The values of tidal volume were related to body weight (No values of tidal volume were recorded for the esophageal pressure change of 4 5± 0 25 cmH_2O)

Means±S D for the tidal volumes of each esophageal pressure group were calculated and plotted graphically to form the pressure volume curve of the chestwall In some infants a pressure transmission of 4–5 cmH_2O to the esophagus was not reached, and therefore, the upper part of the *P/V* curve is derived from fewer data points Linear regression analysis was performed between V_T and change in *Pes* The slope of this regression line represents the chestwall compliance The pressure volume curves for the premature and full term infants were analysed separately, and the slopes of the curves were compared statistically Similarly, individual values for *Cw* were determined and plotted against the infant s gestational age

RESULTS

Results are given in Table 1 and Fig 2 In both premature and in full-term infants there is a linear increase in esophageal pressure change with increasing tidal volume (r=0 95 and 0 79 respectively) The slope of the curves is significantly flatter in the full term infants, indicating that their chestwall compliance is lower Mean chestwall compliance in the premature infants was 6 4, while in the full term infants it was 4 2 $ml/(cmH_2O \times kg)$ (p<0 001)

Chestwall compliance was similar in premature infants with patent ductus arteriosus and hyaline membrane disease The decrease in

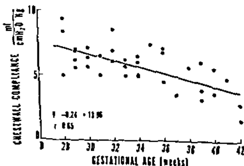


Fig 3 Relation between gestational age and chestwall compliance in 36 newborn infants

Table 1 *Tidal volume[a] (ml/kg) and esophageal pressures in full term and premature infants*

Esophageal pressure (cmH_2O)	0 25–0 75	0 75–1 25	1 25–1 75	1 75–2 25	2 25 2 75	2 75–3 [illegible]5
Tidal volume (ml/kg) in full term newborns	2 2±0 6	4 1±1 5	6 1±2 1	8 0±2 9	10 0±3 9	1[illegible] 2+3 9
Tidal volume (ml/kg) in premature newborns	3 7±1 0	6 6–1 7	10 1±2 7	13 1±3 1	17 1±3 6	20 0+4 1

[a] Values for tidal volume are given as means ± S D

Cw with advancing gestational age was linear and progressive, as shown in Fig 3. According to the regression line *Cw* is 7 2 ml/(cmH_2O ×kg) at 28 weeks gestation and decreases to 3 9 at 42 weeks gestation

A poor correlation was found between airway pressure and change in esophageal pressure in the premature infants (r=0 52) (Table 2) The application of an airway pressure of 20 cmH_2O in a premature infant with patent ductus arteriosus may result in a 3 5–4 0 cmH_2O pressure transmission to the esophagus, whereas only 1 cmH_2O transmission could be recorded in some infants with hyaline membrane disease If the degree of airway pressure transmitted to the esophagus is calculated separately for infants with hyaline membrane disease and patent ductus arteriosus the correlation improves considerably (Table 2 and Fig 4) In the patients with patent ductus arteriosus, 12% of the pressure was transmitted to the esophagus (r=0 76) whereas in patients with hyaline membrane disease only 5% was transmitted (r=0 79) (Fig 4)

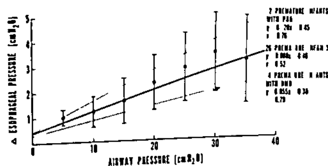


Fig 4 Relation between esophageal and airway pressure in all 26 premature infants in the 12 infants with PDA and the 14 with HMD (Mean ± S D of the esophageal pressure in the 26 premature infants are shown)

DISCUSSION

There is little information about *Cw* in newborns Richards & Bachman (17) and Nightingale & Richards (14) determined the total static compliance of the respiratory system in infants below 6 months of age who were paralysed with succinylcholine or *d* tubocurare Because their values for static compliance of the respiratory system were similar to those [illegible]

Reynolds & Etstan (16) determined *Cw* directly in 15 infants and arrived at a value of about 4 times that of normal lung compliance given in the literature for infants of similar weight

Pressure volume curves of the chestwall were determined indirectly in adult and newborn dogs by Agostoni (2) and in goats by Avery & Cook (3) Chestwall compliance was markedly higher in the newborn than in the adult animals and the pressure volume curve showed a linear course over most of the tidal volume range These findings are consistent with the observations made in our patients

We are expanding these previous observations by showing that the *Cw* of the newborn is related to gestational age the more premature the infant is the higher is his *Cw*

1 When a given pressure is applied to the airway (*P*aw) under static conditions and with the respiratory muscles relaxed by mechanical hyperventilation *P*aw is partly used to overcome the elastic and surface forces of the lung (P_L) and is partly used to move the chestwall (*P*pl) to a higher thoracic volume In the adult, at midrange volumes, the lung and chestwall have a similar compliance (9) and

25–3 75	3 75–4 25	4 75–5 25
4 4±6 0	16 4±7 2	
2 5+3 2	25 0±4 0	31 0±4 2

Paw is therefore divided into similar portions to expand the lungs and move the chestwall ($Paw=P_L+Ppl$) In the newborn the chestwall is much more compliant than the lung If a normal C_L for premature infants of 1 2 ml/($cmH_2O \times kg$) is assumed (5, 7), Cw is about 5 times higher In the full term neonate, Cw is about 3 times higher than C_L, assuming a normal C_L of 1 4 ml/($cmH_2O \times kg$) (10, 18) (Fig 3) This finding indicates that in premature infants under normal conditions only 1/6 of Paw will be necessary to move the chestwall and will be transmitted to the esophagus, in the full term, 1/4 of Paw will be transmitted to the esophagus

If large tidal volumes are used, the proportion of Paw transmitted to the esophagus will be even smaller because the C_L will decrease due to overdistension of the alveoli This will necessitate a higher than proportional increase in P_L for a given change in lung volume, whereas Pes will increase proportionally to V_T because of the linear P/V relationship of the chestwall

The fraction of Paw transmitted to the esophagus is greatly diminished in cases of decreased C_L produced by disease In the group of premature infants with hyaline membrane disease only 5% was transmitted, while in the patients with patent ductus arteriosus 12% transmission was found With decreased C_L the largest part of Paw applied is used to distend the lung This is the reason why the correlation between Paw and Pes which is influenced by C_L improves if patients with similar C_L are grouped for analysis

The results indicate that Pes is well correlated with V_T, but only indirectly correlated with Paw Using a tidal volume of 10 ml/kg a Pes of about 1 5 cmH_2O can be expected in the premature and 2 5 cmH_2O in the full-term, irrespective of the Paw used The fraction of Paw transmitted to the esophagus, therefore, varies widely with changes in C_L

2 One of the detrimental side effects of mechanical ventilation and continuous positive airway pressure is a decrease in cardiac output secondary to a decrease in central venous return because of increased intrathoracic pressure (1,13) In the premature infant with a very compliant chestwall and a C_L which is frequently decreased, the transmission of Paw to the pleural space will be only 5–10%, and therefore, the effects on cardiac output are probably small In the full term newborn with normal lungs, 25% of Paw may be transmitted to the pleural space and therefore the use of high airway pressures in these patients may result in a significant effect on cardiac output (The effect will be even more pronounced in the adult) The high Cw of the newborn protects the intrathoracic vascular structures from a large increase in Ppl and from the consequent decrease in central venous return

3 The high Cw limits the outward recoil of the chest, which may become ineffective in counteracting the retractive forces of the lung

Table 2 *Airway and esophageal[a] pressure in different groups of premature infants*

Airway pressure (cmH_2O)	5	10	15	20	25	30	35
Esophageal pressure (cmH_2O)							
26 premature infants	1 0±0 3	1 2±0 6	1 6±0 9	2 3±1 1	2 9±1 4	3 5±1 6	3 2±1 8
12 premature infants with PDA	1 1±0.3	1.6±0 6	2 2±1 0	2 8±1 2	3 4±1 6	4 1±1.8	4 4±1 8
14 premature infants with HMD	0.5±0 1	0 8±0 2	1 0±0 3	1 6±0 4	1 7±0 4	2 3±0 4	2 0±0 7

[a] Values for esophageal pressure are given as means ± S D

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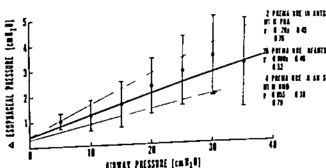


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Acta Pædiatr Scand 69 365–370, 1980

UMBILICAL ARTERY CATHETERIZATION IN NEWBORNS

IV Strain-Gauge Plethysmography for the Diagnosis of Catheter-Related Thrombo-Embolism in the Legs

GÖRAN WESSTROM and CLAES LASSVIK

From the Departments of Paediatrics and Clinical Physiology, University Hospital, Linkoping, Sweden

ABSTRACT Wesstrom, G. and Lassvik, C. (Departments of Paediatrics and Clinical Physiology, University Hospital, Linkoping, Sweden). Umbilical artery catheterization in newborns. IV. Strain gauge plethysmography for the diagnosis of catheter-related thrombo-embolism in the legs Acta Paediatr Scand, 69 365, 1980.—Strain gauge plethysmography was performed shortly after withdrawal of the catheter in 48 of 49 newborn infants who had undergone umbilical artery catheterization. Plethysmography was used to find a non-invasive method for diagnosing catheter related thrombo-embolism in neonates. The results were compared with the findings at angiography. The method permits simultaneous measurements of the systolic blood pressure and of the resting and submaximal blood flow in both legs Nine infants showed angiographic signs of total or partial occlusion in one leg, and 8 of them were investigated with plethysmography. The systolic blood pressure was significantly reduced in infants with total and/or partial occlusion, but the blood flow was reduced only in infants with total occlusion The peak flow after suprasystolic occlusion was significantly oftener delayed in infants with thrombo-embolism in the leg.

KEY WORDS Newborn infant, umbilical artery, catheterization, thrombo-embolism, strain gauge plethysmography

Thrombo-embolism is a well known complication following umbilical artery catheterization (12, 15, 20, 22) The diagnosis is probably frequently overlooked as clinical signs are often absent (20, 22) In the past, the diagnosis has mainly been established by angiography (9, 20, 22) or at necropsy (12, 15), and the incidence of thrombo-embolism has been given as 24–95% and 3 5–48%, respectively So far no simple, reliable, non-invasive method for the diagnosis of circulatory disturbances in infants after umbilical artery catheterization has been used Strain-gauge plethysmography, first described by Whitney (1953) in adults, has however been shown to be suitable for determining arterial blood pressure and blood flow in neonates (2, 5, 13, 17) and for diagnosing thrombo-embolism in the legs after percutaneous catheterization of the femoral arteries in children (19) We therefore felt that this technique might prove useful for the diagnosis of thrombo-embolism after umbilical artery catheterization The present study was therefore designed to compare the results of strain-gauge plethysmography with the findings at angiography in a series of infants who had undergone umbilical artery catheterization, and in particular to find out whether non-invasive determination of blood pressure and blood flow, as done with the plethysmographic technique, could be helpful in the diagnosis of thrombo-embolism after such catheterization.

MATERIAL

The primary series consisted of 71 sick newborn infants with an indwelling umbilical artery catheter treated during a 19-month period (for details of the series, indications for catheterization, and types, positions, and management of the catheters, see Wesstrom, 22) Of these infants 49 were investigated with angiography, 48 of them were also examined with strain gauge plethysmography in the

(2) This will result in a decrease in functional residual capacity and ventilation will take place within the range of the closing volume (2), leading to alveolar collapse and ventilation/perfusion inequalities observed especially in premature infants (15, 20) These disturbances can be immediately improved by applying negative pressure around the chest, thus increasing the outward recoil of the chest-wall (19)

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Submitted June 12 1979
Accepted Oct 24 1979

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Table 2 *Systolic blood pressure and blood flow differences between the legs in infants with unilateral ileo-femoral occlusion compared with infants without occlusion*

The differences are expressed both in absolute and relative values Mean and S D

	Systolic blood pressure difference		Resting blood flow difference		Submaximal blood flow difference	
	mmHg	%	ml/min/ 100 ml tissue	%	ml/min/ 100 ml tissue	%
Total occlusion	31 3*** ±7 5	42 8*** ±9 2	2 7 n s ±2 7	68 5* ±56 2	5 7 n s ±3 7	95 1*** ±57.3
Partial occlusion	14 8*** ±7 4	19 4*** ±13 9	0 8 n s ±1 5	19 8 n s ±28 8	2 3 n s ±2 4	32 4 n s ±34 5
Total and partial occlusion	21 0*** ±11 0	28 3*** ±16 9	1 6 n s ±1 9	39 0 n s ±41 1	3 7 n s ±3 1	56 3** ±51 4
No occlusion	4 4 ±3 9	5 3 ±4 5	1 0 ±1 3	26 7 ±27 5	3 1 ±3 1	25 1 ±21 3

*$p<0.05$ **$p<0.01$ ***$p<0.001$ n s = no significance, in comparison with the group without occlusion

were compared in infants with total and/or partial unilateral ileofemoral occlusion and in infants showing normal angiograms (Table 2) The mean difference in systolic blood pressure between the legs was significantly greater in infants with total and/or partial occlusion, both in absolute and relative values ($p<0.001$) *Concerning differences in resting and submaximal blood flow*, the relative values in infants with total occlusion differed significantly from those in infants without occlusion ($p<0.05$ and $p<0.001$)

Table 3 shows the presence of occlusion in the legs in relation to a set of critical limits of differences in blood pressure, and in resting and submaximal flow between the legs These limits were chosen in order to obtain approximately the same number of cases as there were cases of arterial occlusion in the series Seven infants showed a blood pressure difference of at least 15 mmHg, and 6 of these had thrombo-embolism This incidence of thrombo-embolism differed significantly ($p<0.001$) from that found in the remaining infants (2 of 36) With regard to resting and submaximal flow, the incidence of thrombo embolism was not significantly increased in infants showing marked flow differences between the legs,

Table 3 *The incidence of thrombo-embolism in the legs related to a set of critical limits in variables of diagnostic interest*

Tests of significance are one tailed Fisher exact tests

Diagnostic criteria	Relative incidence of thrombo-embolism among infants fulfilling the criteria		Relative incidence of thrombo-embolism among infants not fulfilling the criteria
Difference in blood pressure between the legs ≥15 mmHg	6/7=86%	$p<0.001$	2/36=6%
Difference in resting flow between the legs ≥2 ml/min/100 ml tissue	2/8=25%	n s	6/27=22%
Differences in submaximal flow between the legs ≥4 ml/min/100 ml tissue	3/8=38%	n s	5/25=20%
Delayed peak flow	4/7=57%	$p<0.05$	4/25=16%

Table 1. *Comparison between infants with and without catheter-related thrombo-embolism with regard to birth weight, gestational age, Apgar score at 5 min, and duration of catheterization*

Mean and S D

	Infants with thrombo-embolism (*n*=14)	Infants with thrombo-embolism in the legs (*n*=9)	Infants without thrombo-embolism (*n*=35)	Total number of infants (*n*=49)
Birth weight (g)	2 180±556	2 223±582	2 125±942	2 141±844
Gestational age (weeks)	35 4±3 3	35 7±2 5	34 4±3 6	34 7±3 5
Apgar score (5 min)	8 0±2 2	8 4±1 4	7 9±2 1	7 9±2 1
Duration of catheterization (hours)	81 8±42 8	91 3±38 6	57 3±27 3	64 3±33 9

neonatal period Thirteen died before investigations could be performed, and in the remaining 9 angiography was not done or could not be evaluated

Details of birth weight, gestational age, Apgar score at 5 min, and duration of catheterization are given in Table 1

METHODS

One dimensional angiography was performed by manual injection of 2–5 ml 45% Urografin® while the catheter remained in situ Thrombo-embolism was considered to be present when angiography disclosed total or partial occlusion of the aorta or a pelvic or leg artery, or an obvious sheath round the catheter

The crural circulation was examined within 4 days of removal of the catheter in most cases The infant lay supine in an incubator, with the knees slightly flexed A mercury in rubber strain gauge plethysmograph was used to measure the systolic blood pressure and the blood flow (5, 18, 23)

The strain gauge was wrapped round the thickest part of each calf, and connected to a balanced Wheatstone bridge with electrical volume calibration (Siemens Elema) An occlusion cuff (3×20 cm) was placed round each thigh just above the knee and connected to an infratone OS22 (Siemens Elema) for semiautomatic inflation and deflation of the cuff Increase in leg volume distends the gauge producing increase in the electrical resistance (5, 23) The volume changes were recorded on a Mingograph 61 (Siemens Elema) Care was taken to ensure symmetry of cuffs and strain gauges and of the legs Both legs were examined simultaneously, and all results were expressed as differences between the legs

The following measurements were made

Systolic blood pressure Both cuffs were inflated to a suprasystolic pressure and then slowly deflated The systolic blood pressure was defined as the deflection point where calf volume started to increase owing to arterial inflow (4, 7 17) The pressure was calculated from the mean values of 3 readings

Blood flow at rest was measured as the increase in calf volume under venous occlusion with a cuff pressure of 40 mmHg (1 3, 13) The flow was calculated from the means of 3 readings, and expressed in ml/min/100 m tissue

an occlusion pressure of 40 mmHg The first registratio

not occur on the first recording delayed peak flow wa said to be present

RESULTS

Angiography In 14 of the 49 infants thrombo embolism was diagnosed at angiography The ileo-femoral artery was totally occluded in 3 and partially occluded in 5 In 5 infants the thrombus was located round the catheter in the aorta, where it did not seem to influence the blood flow One infant showed total occlusion of the popliteal artery on the contralateral side, whereas in all other infants with thrombo-embolism in the leg, an artery on the catheterized side was affected

The duration of catheterization was longer in infants with thrombo embolism ($p<0\ 05$) than in those without The difference was more pronounced when comparison was restricted to infants with occlusion in the leg ($p<0\ 01$) No differences were found concerning birth weight, gestational age, or Apgar score (Table 1)

Plethysmography The differences between the two legs with regard to systolic blood pressure and resting and submaximal flow

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	Systolic blood pressure difference		Resting blood flow difference		Submaximal blood flow difference	
	mmHg	%	ml/min/ 100 ml tissue	%	ml/min/ 100 ml tissue	%
Total occlusion	31.3*** ±7.5	42.8*** ±9.2	2.7 n s ±2.7	68.5* ±56.2	5.7 n s ±3.7	95.1*** ±57.3
Partial occlusion	14.8*** ±7.4	19.4*** ±13.9	0.8 n s ±1.5	19.8 n s ±28.8	2.3 n s ±2.4	32.4 n s ±34.5
Total and partial occlusion	21.0*** ±11.0	28.3*** ±16.9	1.6 n s ±1.9	39.0 n s ±41.1	3.7 n s ±3.1	56.3** ±31.4
No occlusion	4.4 ±3.9	5.3 ±4.5	1.0 ±1.3	26.7 ±27.5	3.1 ±3.1	25.1 ±21.3

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Table 1. *Comparison between infants with and without catheter-related thrombo-embolism with regard to birth weight, gestational age, Apgar score at 5 min, and duration of catheterization*

Mean and S D

	Infants with thrombo-embolism ($n=14$)	Infants with thrombo-embolism in the legs ($n=9$)	Infants without thrombo-embolism ($n=35$)	Total number of infants ($n=49$)
Birth weight (g)	2 180±556	2 223±582	2 125±942	2 141±844
Gestational age (weeks)	35 4±3 3	35 7±2 5	34 4±3 6	34 7±3.5
Apgar score (5 min)	8 0±2 2	8 4±1 4	7 9±2 1	7 9±2 1
Duration of catheterization (hours)	81 8±42 8	91 3±38 6	57 3±27 3	64.3±33 9

neonatal period Thirteen died before investigations could be performed, and in the remaining 9 angiography was not done or could not be evaluated

Details of birth weight, gestational age, Apgar score at 5 min, and duration of catheterization are given in Table 1

METHODS

One-dimensional angiography was performed by manual injection of 2–5 ml 45% Urografin® while the catheter remained in situ Thrombo-embolism was considered to be present when angiography disclosed total or partial occlusion of the aorta or a pelvic or leg artery, or an obvious sheath round the catheter

The crural circulation was examined within 4 days of removal of the catheter in most cases The infant lay supine in an incubator, with the knees slightly flexed A mercury in rubber strain gauge plethysmograph was used to measure the systolic blood pressure and the blood flow (5, 18, 23)

The strain gauge was wrapped round the thickest part of each calf, and connected to a balanced Wheatstone bridge with electrical volume calibration (Siemens Elema) An occlusion cuff (3×20 cm) was placed round each thigh just above the knee, and connected to an infratone OS22 (Siemens Elema) for semiautomatic inflation and deflation of the cuff Increase in leg volume distends the gauge producing increase in the electrical resistance (5, 23) The volume changes were recorded on a Mingograph 61 (Siemens Elema) Care was taken to ensure symmetry of cuffs and strain gauges and of the legs Both legs were examined simultaneously and all results were expressed as differences between the legs

The following measurements were made

Systolic blood pressure Both cuffs were inflated to a suprasystolic pressure, and then slowly deflated The systolic blood pressure was defined as the deflection point where calf volume started to increase owing to arterial inflow (4, 7, 17) The pressure was calculated from the mean values of 3 readings

Blood flow at rest was measured as the increase in calf volume under venous occlusion with a cuff pressure of 40 mmHg (1, 3, 13) The flow was calculated from the means of 3 readings, and expressed in ml/min/100 tissue

Submaximal blood flow was measured as the peak flow after 4 min of suprasystolic occlusion After releasing the cuff repeated measurements of the flow were made under an occlusion pressure of 40 mmHg The first registration was obtained within ca 15 sec after release of the suprasystolic occlusion, and 3–4 further recordings were made within the first min (2, 6, 18 21) When the peak flow did not occur on the first recording, delayed peak flow was said to be present

RESULTS

Angiography In 14 of the 49 infants thrombo-embolism was diagnosed at angiography The ileo-femoral artery was totally occluded in and partially occluded in 5 In 5 infants the thrombus was located round the catheter in the aorta, where it did not seem to influence the blood flow One infant showed total occlusion of the popliteal artery on the contralateral side, whereas in all other infants with thrombo-embolism in the leg, an artery on the catheterized side was affected

The duration of catheterization was longer in infants with thrombo-embolism ($p<0.05$) than in those without The difference was more pronounced when comparison was restricted to infants with occlusion in the leg ($p<0.01$) No differences were found concerning birth weight, gestational age, or Apgar score (Table 1)

Plethysmography The differences between the two legs with regard to systolic blood pressure and resting and submaximal flow

Table 2 *Systolic blood pressure and blood flow differences between the legs in infants with unilateral ileo-femoral occlusion compared with infants without occlusion*

The differences are expressed both in absolute and relative values Mean and S D

	Systolic blood pressure difference		Resting blood flow difference		Submaximal blood flow difference	
	mmHg	%	ml/min/ 100 ml tissue	%	ml/min/ 100 ml tissue	%
Total occlusion	31 3*** ±7 5	42 8*** ±9 2	2 7 n s ±2 7	68 5* ±56 2	5 7 n s ±3 7	95 1*** ±57 3
Partial occlusion	14 8*** +7 4	19 4*** ±13 9	0 8 n s ±1 5	19 8 n s ±28 8	2 3 n s ±2 4	32 4 n s ±34 5
Total and partial occlusion	21 0*** ±11 0	28 3*** ±16 9	1 6 n s ±1 9	39 0 n s ±41 1	3 7 n s ±3 1	56 3** ±51 4
No occlusion	4 4 ±3 9	5 3 ±4 5	1 0 ±1 3	26 7 ±27 5	3 1 ±3 1	25 1 ±21 3

*$p<0\ 05$ **$p<0\ 01$ ***$p<0\ 001$ n s =no significance in comparison with the group without occlusion

were compared in infants with total and/or partial unilateral ileofemoral occlusion and in infants showing normal angiograms (Table 2) The mean difference in systolic blood pressure between the legs was significantly greater in infants with total and/or partial occlusion, both in absolute and relative values ($p<0\ 001$) Concerning differences in resting and sub maximal blood flow, the relative values in in fants with total occlusion differed significantly from those in infants without occlusion ($p<0\ 05$ and $p<0\ 001$)

Table 3 shows the presence of occlusion in the legs in relation to a set of critical limits of differences in blood pressure, and in resting and submaximal flow between the legs These limits were chosen in order to obtain approximately the same number of cases as there were cases of arterial occlusion in the series Seven infants showed a blood pressure difference of at least 15 mmHg, and 6 of these had thromboembolism This incidence of thromboembolism differed significantly ($p<0\ 001$) from that found in the remaining infants (2 of 36) With regard to resting and submaximal flow, the incidence of thrombo embolism was not significantly increased in infants showing marked flow differences between the legs,

Table 3 *The incidence of thrombo embolism in the legs related to a set of critical limits in variables of diagnostic interest*

Tests of significance are one tailed Fisher exact tests

Diagnostic criteria	Relative incidence of thrombo-embolism among infants fulfilling the criteria		Relative incidence of thrombo-embolism among infants not fulfilling the criteria
Difference in blood pressure between the legs ≥15 mmHg	6/7=86%	$p<0\ 001$	2/36=6%
Difference in resting flow between the legs ≥2 ml/min/100 ml tissue	2/8=25%	n s	6/27=22%
Differences in submaximal flow between the legs ≥4 ml/min/100 ml tissue	3/8=38%	n s	5/25=20%
Delayed peak flow	4/7=57%	$p<0\ 05$	4/25=16%

Table 4 *Differences between the legs with regard to systolic blood pressure and resting and submaximal blood flow in individual infants with thrombo-embolism*

The differences are expressed both in absolute and relative values

Type of thrombo-embolism	No	Blood pressure difference mmHg	Blood pressure difference %	Resting flow difference ml/min/100 ml tissue	Resting flow difference %	Submaximal flow difference ml/min/100 ml tissue	Submaximal flow difference %
A							
Total occlusion of leg artery	1	39	53	1 7	52	5 6	97
	2	24	40	5 7	131	9 4	151
	3	31	35	0 7	22	2 0	37
	4	–	–	–	–	–	–
B							
Partial occlusion of leg artery	5	25	43	0 1	8	3 6	81
	6	16	17	0 4	4	3 2	34
	7	5	6	2 3	41	0 3	1
	8	11	14	1 4	41	5 1	48
	9	17	17	0 2	12	0	0
C							
Catheter thrombosis in aorta	10	7	8	1 2	36	0 6	7
	11	0	0	0 9	24	4 0	32
	12	21	25	0 3	7	2 0	20
	13	7	7	0 7	45	–	–
	14	2	3	–	–	–	–

regardless of the choice of critical limits On comparison of relative values, however, a difference in submaximal flow of 33% or more between the legs proved to be significantly oftener associated with thrombo embolism ($p<0$ 01) A delayed peak flow occurred significantly oftener in infants with thrombo-embolism in the legs ($p<0$ 05)

Plethysmographic data for individual infants with angiographic signs of thrombo embolism are given in Table 4 Infants showing thrombosis round the catheter in the aorta did not differ significantly from infants without thrombosis with regard to any of the criteria studied One infant (no 4) was referred to another hospital and could not be examined with plethysmography In another (no 12), in whom angiography showed thrombosis round the catheter in the aorta, there was a blood pressure difference of 21 mmHg between the legs

DISCUSSION

In the past the diagnosis of thrombo embolism following umbilical artery catheterization has mainly been made by angiography This method has disadvantages, however It is in vasive and involves radiation Follow up in vestigations are difficult once the catheter ha been withdrawn, since percutaneous cathe terization is not without risk With one dimer sional angiography, which is the usual methoc it is often impossible to determine the degre of occlusion Further, the functional status c the peripheral circulation cannot be evaluate by angiography

In the present study strain gauge plethys mography with blood pressure and blood flov determinations was compared with angio graphy in diagnosing thrombo embolism, sinc in occlusive arterial disease the systolic bloo pressure and the blood flow are reduced dista to the lesion (7, 18)

Strain gauge plethysmography is easy t perform even in an incubator, both legs can be investigated simultaneously, and the method is safe The simplicity in handling makes the strain gauge technique superior to other forms of plethysmography such as those using water or air filled tourniquets (4, 14) By using simul taneous measurements of both legs under

identical circumstances the results can be expressed as differences between the legs To detect occlusions in both legs or the aorta it is necessary to compare the blood pressure between arm and leg The catheter was withdrawn between angiography and plethysmography The act of withdrawal may strip off a catheter thrombus and cause occlusion of a distal artery either in the ipsilateral or contralateral leg since it has been shown that contralateral occlusion can occur (20, 21)

The systolic blood pressure measured with strain gauge plethysmography tallies closely with intra arterially measured blood pressure, and the method allows measurements in the absence of arterial pulsations when the pressure is very low (4, 8, 11) The Doppler ultrasonic technique has the same advantages, but does not readily permit simultaneous measurements in the legs and does not allow measurements of blood pressure and blood flow with the same equipment (10, 25) Occlusion in a branch artery cannot be detected with plethysmography in which the legs are investigated segmentally but the Doppler technique, in which the arteries are investigated individually will disclose such a lesion A certain degree of occlusion is needed to give a reduction in the systolic pressure When a stenosis exceeds ca 50% of the arterial diameter a pressure gradient can be demonstrated (24)

Measurement of resting blood flow is of limited value in diagnosing thrombo-embolism in the ileo femoral artery, as an occlusion will not influence the blood flow until it is sufficient to cause flow resistance equal to or exceeding the peripheral flow resistance At rest, when the peripheral resistance is high, this requires a 80–90% reduction in the lumen of the artery After a period of circulatory arrest the peripheral resistance falls, and narrowing of the artery will have a relatively greater effect on the flow This explains the advantage in determining submaximal flow instead of resting flow when diagnosing thrombo embolism (19) A disadvantage with measuring submaximal blood flow is the high incidence of technical failures as it is of course impossible to get the infant patients to cooperate

In the present study good correlation was obtained between systolic blood pressure differences and thrombo embolism in the leg as diagnosed by angiography A difference in systolic blood pressure of 15 mmHg or more was consistent with unilateral occlusion in most cases This is in accordance with a methodological study on healthy newborn infants in which we found that the systolic blood pressure difference between the legs was less than 10 mmHg in all cases (16) However, two infants with angiographic signs of occlusion showed blood pressure differences between the legs of less than 15 mmHg one (no 7, Table 4) had a branch occlusion at the origin of the internal iliac artery and the other (no 8) a partial occlusion of the common iliac artery In the infant (no 12) with a catheter thrombosis in the aorta at angiography and a blood pressure difference of 21 mmHg at plethysmography, the thrombus might have been stripped off the catheter when this was withdrawn, subsequently causing occlusion in the uncatheterized leg

Both submaximal and resting blood flow were found to be significantly reduced only on comparing relative values and only in patients in whom the main artery was totally occluded Delayed peak flow occurred significantly oftener in infants with occlusion in the leg, but in most instances other signs of occlusion were present Thus the flow determinations were of limited value in diagnosing thrombo-embolism in the leg, and had no advantages over the blood pressure measurements

In conclusion, our study has shown that the systolic blood pressure as measured by strain-gauge plethysmography is a simple, harmless, reliable, non invasive method for the diagnosis of thrombo embolism in the leg following umbilical artery catheterization

ACKNOWLEDGEMENT

This investigation was supported by grants from Forenade Liv Mutual Group Life Insurance Company and Pre natalforskningsfonden

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Submitted Aug 28 1979
Accepted Oct 19 1979

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Acta Pædiatr Scand 69 371–376, 1980

UMBILICAL ARTERY CATHETERIZATION IN NEWBORNS

V A Clinical Follow up Study

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ABSTRACT Wesstrom, G (Departments of Paediatrics and Clinical Physiology, University Hospital, Linkoping, Sweden) Umbilical-artery catheterization in newborns V A clinical follow up study Acta Paediatr Scand, 69 371, 1980.—The long term effects of umbilical artery catheterization were studied in forty nine children At the age of about 18 months their gross motor and neurological development, peripheral circulation, and growth of the legs were investigated The infants with catheter related thrombo-embolism in the legs diagnosed in the neonatal period with the aid of angiography and investigated by plethysmography were re-examined once or more during the first year of life by strain-gauge plethysmography One child who showed total occlusion of the iliac artery as newborn, had a difference in calf circumference at the 18-month examination of 1.5 cm, the only finding in this study related to catheter associated thrombosis In infants with neonatal thrombo-embolism in the legs the initial crural difference in systolic blood pressure diminished slowly, and at 12 months a blood pressure difference of more than 10 mmHg remained in only one infant

KEY WORDS Newborn infant, umbilical artery, catheterization thrombo-embolism, strain-gauge plethysmography

Umbilical artery catheterization is used routinely in the supervision of sick newborn infants when repeated arterial acid base samples are necessary Umbilical artery catheters have been blamed for thrombo-embolism ischaemic necrosis of abdominal viscera vascular perforation, haemorrhage, and infection (8 16 18 20, 24) Thrombo-embolism seems to be their principal complication, with a reported incidence of 3 5–48% as demonstrated by necropsy and 24–95% as shown by angiography (7, 8, 14, 18) Nevertheless surprisingly few cases of persistent circulatory symptoms have been seen as complications of umbilical artery catheterization This is in contrast to thrombo-embolism following femoral artery catheterization, after which circulatory disturbances and shortness and narrowness of the leg are fairly common, especially in infants and young children (2, 12, 17)

Our earlier angiographic and plethysmographic studies on thrombo embolism following umbilical artery catheterization (25, 26) showed a high incidence of catheter related thrombo embolism on angiography (26%), and 7 of 8 examined infants with occlusion of leg arteries had blood pressure or blood flow anomalies demonstrated by plethysmography

The present study is a continuation of the earlier ones, and was done, to investigate all neonatally catheterized children at 18 months of age with regard to gross motor and neurological development, circulation and growth of the legs, and to re examine in more detail the peripheral circulation in infants with crural thrombo-embolism using strain gauge plethysmography

MATERIAL

During a 19-month period 71 sick newborn infants were subjected to umbilical artery catheterization Of these 49

Table 1. *Comparison between infants with and without catheter-related thrombo-embolism with regard to birth weight, gestational age, Apgar score at 5 min, and duration of catheterization*

Mean and S D

	Infants with thrombo-embolism (n = 14)	Infants with thrombo-embolism in the legs (n = 9)	Infants without thrombo-embolism (n = 35)	Total number of infants (n = 49)
Birth weight (g)	2 160±556	2 223±582	2 125±942	2 141±844
Gestational age (weeks)	35 4±3 3	35 7±2 5	34 4±3 6	34 7±3 5
Apgar score (5 min)	8 0±2 2	8 4±1 4	7 9±2 1	7 9±2 1
Duration of catheterization (hours)	81 8±42 8	91 3±38 6	57 3±27 3	64 3±33 9

were investigated by contrast radiography via the umbilical catheter, and in 48 of the 49 strain gauge plethysmography was also done shortly after the removal of the catheter All 49 infants were investigated at about 18 months of age (range 17–28 months) The material is presented in Table 1 with regard to the occurrence and localization of thrombo-embolism, birth weight, gestational age, Apgar score at 5 min, and duration of catheterization

Nine infants with angiographic signs of ileo-femoral or popliteal artery occlusion and three with normal angiograms but with an intercrural difference in systolic blood pressure of more than 10 mmHg were re examined by strain gauge plethysmography once or more during the first year of life

METHODS

The 18 month examination

The parents were questioned about signs of leg pains, noticeable difference in movements between the legs, or limping and for the developmental milestones such as time for sitting up standing up and walking with and without support These dates were established taking into account the duration of gestation

Neurological examination of the legs was performed including estimation of gross motor development and ability to stand, walk, and run Muscle tone, pain sensitivity at different levels in the lower extremities, and the patellar, achilles and Babinski responses were tested with special emphasis on symmetry

The tibial length and the circumferences of the thigh and calf were measured with a tape measure the results being expressed as difference between the legs The means of 3 measurements were used The tibia was measured from the medial knee joint space to the distal border of the medial malleolus, special care being taken to arrange the legs symmetrically The thigh circumference was measured 7 cm above the patella, and the maximum calf circumference was recorded The tape was kept in firm contact with the skin but was not allowed to compress the tissues

The femoral and dorsalis pedis pulses on each side were palpated and compared The systolic blood pressure in the legs and in the right arm were measured by the Dopp ultrasonic technique (10, 27) The occlusion cuff w placed round the thigh just above the knee and round t upper arm, respectively Standard blood pressure cuffs appropriate size were used (11) The results were e pressed in pressure difference between the two legs a between the unoccluded or uncatheterized leg and t right arm The measurements were performed with t child quietly sitting on the parent s knee The systo blood pressure was calculated as the mean of 3 measur ments

Reexamination with strain gauge plethysmography

[illegible]

The infants lay supine and were lightly sedated (5 n Diazepam rectally) All registrations were made with tl cuff round the thigh, with one exception (see below where the cuff was placed round the calf Both legs we examined simultaneously and the result was expresse as the difference between the legs Details of the metho are given elsewhere (26)

RESULTS

The 18-month examination

Divergent findings at the 18-month examination are given in Table 2 Two children, one with thrombo-embolism in the leg and the other with thrombosis round the catheter in the aorta, were found to have cerebral palsy, and this was subsequently confirmed One of these children with choreo athetosis showed delayed gross motor performance, and also reduction of 1 5 cm in calf circumference and no palpable pulse in the dorsalis pedis artery on the affected side In one child, with a differ-

Table 2 Results of the 18-month examination

	Number of children	Delayed gross motor development	Pathological neurological signs	Difference in thigh circumference between the legs ≥1 cm	Difference in calf circumference between the legs ≥1 cm	Difference in tibia length between the legs ≥1 cm	Difference in blood pressure between the legs ≥10 mmHg
Infants with occlusion in the legs	9	1	1	0	1	1	0
Infants with thrombosis round the catheter in aorta	5	0	1	1	1	0	0
Infants with no thrombo-embolism	35	1	0	1	0	0	0

ence in tibial length of 1 cm, the longer leg was that previously affected by occlusion In all infants the difference in blood pressure between the legs was less than 10 mmHg In none was the blood pressure higher in the arm than in the leg The pulses were equal in all infants but one (see above)

Re examination with strain-gauge plethysmography

At follow up examination of infants with crural thrombo-embolism, the differences in systolic blood pressure between the legs diminished with time, and within 12 months all but one showed a difference of 10 mmHg or less (Fig 1) One infant, referred to another hospital and therefore not examined until 3 months of age, had had occlusion of the popliteal artery in the neonatal period, and the pressure was therefore recorded on the calf, distal to the occlusion

In the 3 infants showing normal angiograms but with an initial blood pressure difference exceeding 10 mmHg the difference fell to less than 10 mmHg within 2 months

It was often impossible to measure blood flow at the follow up examination owing to difficulty in keeping the infant quiet in spite of sedation In the 3 infants who had shown total occlusion of the iliac artery, however, the submaximal flow measurements were successful both initially and at re examination at 3 months of age The reduced flow quickly returned to normal, and in 2 of 3 infants the flow was considerably higher in the previously occluded leg at follow-up

DISCUSSION

The effects of thrombo-embolism following umbilical-artery catheterization have long been a source of worry The serious immediate consequences are well described (8, 13, 18), but the long term effects remain largely unknown since only a few studies have dealt with these problems (3, 19, 22) Boros et al. (3) are the only previous workers who have reexamined children with radiologically confirmed neonatal thrombo-embolism It is well known that infants with a thrombus totally occluding a leg artery do not necessarily show clinical signs (18, 25) The high incidence of thrombo-embolism in the legs discovered at

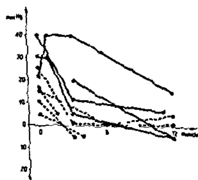


Fig 1 Intercrural systolic blood pressure difference in infants with total (—) and partial (-) occlusion of a leg artery during the first year of life

necropsy (14) or demonstrated by angiography (18) led to the suspicion that clinically inapparent catheter-related thrombo embolism might nevertheless give rise to late significant circulatory disturbance and inhibition of growth in the affected leg Follow-up examinations of infants and children who underwent percutaneous femoral artery catheterization have indeed shown such changes (2, 12)

The present study was aimed at evaluating the possible long term effects of umbilical-artery catheterization and in particular the effects of catheter-related thrombo embolism Neurological examination showed 2 children to have cerebral palsy which cannot have had any connection with the catheter-related thrombus formation—thrombo embolism can give neurological signs of peripheral but not of central type

The effect of altered blood flow on the subsequent growth of an extremity is well documented (5) Inequality in the longitudinal growth of the lower extremities following femoral artery catheterization has been reported (2, 17, 21) Thus, Bassett et al (2) using an orthoroentgenographic technique, found 10 of 28 children to have an intercrural difference of 8 mm or more The failure of growth occurred primarily in the tibia When longitudinal growth was affected, the circumference of the thigh and calf was also reduced Others, using a tape measure, have reported a low incidence of leg length discrepancy however (21) The radiological technique is of course more accurate, but the radiation is obviously a disadvantage The tape measure is probably accurate enough (21)

The discrepancy of 1 5 cm in calf circumference in one infant was probably the only finding concerning growth in this study that was related to a catheter associated thrombus formation In one child in whom a catheter thrombus in the aorta was shown by angiography, the thigh and the calf circumferences differed by 1 cm The thrombus may possibly have been stripped off the catheter during withdrawal, resulting embolus giving rise to subsequent discrepancy in growth This in unlikely, however, as no blood pressure difference between the legs was found either initially or at re examination

The systolic blood pressure in the legs was used to evaluate the state of the peripheral circulation, since the pressure is reduced distal to an arterial occlusion (6) At the 18 month examination the Doppler ultrasonic technique was applied This has been successfully used in the diagnosis of thrombo embolism after femoral artery catheterization and for evaluating occlusive arterial disease in adults (1 28) It is simple and requires little equipment

The intercrural difference in systolic blood pressure was within the normal range, and less than 10 mmHg (3, 15) in all children This indicates recanalization of the artery Arm and leg blood pressures were compared in order to disclose aortic or bilateral leg thrombo-embolism All children showed higher blood pressure in the legs, however

The result of the 18 month study confirms that of Boros et al (3), who found no positive correlations between angiographic signs of thrombo embolism in the neonatal period and intercrural length, circumference, or blood pressure differences at 4 years of age

Re examination of the circulation in infants with thrombo embolism was done by strain gauge plethysmography, which is non invasive and safe and fairly easy to handle, and which permits examination of both legs simultaneously (4, 19 26) A high rate of technical failures in recording the flows owing to the natural lack of cooperation reduced the value of this part of the examination The systolic blood pressure was also more reliable than the blood flow in diagnosing crural thrombo embolism in the neonatal period A pressure difference of 15 mmHg or more indicated unilateral occlusion in most cases (26) On re examination during the first year the difference returned to normal in all but one infant In the 3 in whom the determination of blood flow was successful at re examination the submaximal flow quickly increased in the previ

ously occluded leg, the flow became even higher than in the normal leg in 2 of the 3, even though the blood pressure remained lower on that side Return in the blood pressure and flow to normal in an occluded artery probably requires a combination of activation of the fibrinolytic system and recruitment of collateral vessels In an earlier study we found the fibrinolytic activity in the arterial wall to be within the normal range in this age group even in infants with thrombosis (9). The rapid return to normal blood flow probably mainly depends on increase in the collateral flow (17)

Catheterization of the umbilical artery with or without thrombo-embolism seems to have only slight long-term effects on leg circulation and growth The observation time in this study is short, however, and it cannot be excluded that thrombo embolism may give rise to the precocious appearance of local arteriosclerotic lesions (17, 23) In spite of the almost complete absence of sequelae in the present study, catheterization of the umbilical artery is not without risk, and careful selection of infants for this procedure is essential

ACKNOWLEDGEMENT

This investigation was supported by grants from Forenade Liv Mutual Group Life Insurance Company and from Prenatalforskningsfonden

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necropsy (14) or demonstrated by angiography (18) led to the suspicion that clinically inapparent catheter related thrombo embolism might nevertheless give rise to late significant circulatory disturbance and inhibition of growth in the affected leg Follow up examinations of infants and children who underwent percutaneous femoral artery catheterization have indeed shown such changes (2, 12)

The present study was aimed at evaluating the possible long term effects of umbilical artery catheterization and in particular the effects of catheter related thrombo embolism Neurological examination showed 2 children to have cerebral palsy which cannot have had any connection with the catheter related thrombus formation—thrombo embolism can give neurological signs of peripheral but not of central type

The effect of altered blood flow on the subsequent growth of an extremity is well documented (5) Inequality in the longitudinal growth of the lower extremities following femoral artery catheterization has been reported (2, 17, 21) Thus, Bassett et al (2) using an orthoroentgenographic technique, found 10 of 28 children to have an intercrural difference of 8 mm or more The failure of growth occurred primarily in the tibia When longitudinal growth was affected, the circumference of the thigh and calf was also reduced Others using a tape measure, have reported a low incidence of leg length discrepancy however (21) The radiological technique is of course more accurate, but the radiation is obviously a disadvantage The tape measure is probably accurate enough (21)

The discrepancy of 1 5 cm in calf circumference in one infant was probably the only finding concerning growth in this study that was related to a catheter associated thrombus formation In one child in whom a catheter thrombus in the aorta was shown by angiography the thigh and the calf circumferences differed by 1 cm The thrombus may possibly have been stripped off the catheter during withdrawal, resulting embolus giving rise to subsequent discrepancy in growth This in unlikely, however, as no blood pressure difference between the legs was found either initially or at re examination

The systolic blood pressure in the legs was used to evaluate the state of the peripheral circulation, since the pressure is reduced distal to an arterial occlusion (6) At the 18 month examination the Doppler ultrasonic technique was applied This has been successfully used in the diagnosis of thrombo embolism after femoral artery catheterization and for evaluating occlusive arterial disease in adults (1, 28) It is simple and requires little equipment

The intercrural difference in systolic blood pressure was within the normal range, and less than 10 mmHg (3, 15) in all children This indicates recanalization of the artery Arm and leg blood pressures were compared in order to disclose aortic or bilateral leg thrombo embolism All children showed higher blood pressure in the legs however

The result of the 18 month study confirms that of Boros et al (3) who found no positive correlations between angiographic signs of thrombo embolism in the neonatal period and intercrural length, circumference or blood pressure differences at 4 years of age

Re examination of the circulation in infants with thrombo embolism was done by strain gauge plethysmography which is non invasive and safe and fairly easy to handle and which permits examination of both legs simultaneously (4 19 26) A high rate of technical failures in recording the flows owing to the natural lack of cooperation reduced the value of this part of the examination The systolic blood pressure was also more reliable than the blood flow in diagnosing crural thrombo embolism in the neonatal period A pressure difference of 15 mmHg or more indicated unilateral occlusion in most cases (26) On re examination during the first year the difference returned to normal in all but one infant In the 3 in whom the determination of blood flow was successful at re examination the submaximal flow quickly increased in the previ

ously occluded leg, the flow became even higher than in the normal leg in 2 of the 3, even though the blood pressure remained lower on that side Return in the blood pressure and flow to normal in an occluded artery probably requires a combination of activation of the fibrinolytic system and recruitment of collateral vessels In an earlier study we found the fibrinolytic activity in the arterial wall to be within the normal range in this age group even in infants with thrombosis (9). The rapid return to normal blood flow probably mainly depends on increase in the collateral flow (17)

Catheterization of the umbilical artery with or without thrombo-embolism seems to have only slight long-term effects on leg circulation and growth The observation time in this study is short, however, and it cannot be excluded that thrombo embolism may give rise to the precocious appearance of local arteriosclerotic lesions (17, 23) In spite of the almost complete absence of sequelae in the present study, catheterization of the umbilical artery is not without risk, and careful selection of infants for this procedure is essential

ACKNOWLEDGEMENT

This investigation was supported by grants from Forenade Liv Mutual Group Life Insurance Company and from "Prenatalforskningsfonden

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Acta Pædiatr Scand 69 377–383, 1980

TRANSEPIDERMAL WATER LOSS IN NEWBORN INFANTS

IV. *Small for Gestational Age Infants*

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ABSTRACT Hammarlund, K. and Sedin, G. (Department of Paediatrics, University Hospital, Uppsala, Sweden) Transepidermal water loss in newborn infants IV. Small for gestational age infants Acta Paediatr Scand, 69 377, 1980.—Using a method described earlier, the evaporation rate (ER) from the skin was studied at different ambient humidities in 14 full term and 10 pre-term small for gestational age (SGA) infants Transepidermal water loss (TEWL) was estimated in 25 SGA infants born after 30–40 weeks of gestation. Comparisons were made with infants appropriate for gestational age (AGA) A linear relationship was found between ER and ambient humidity in full term SGA infants, but with lower ER values than in AGA infants Lower ER values were also found in moderately pre-term SGA infants at different ambient humidities ER was higher at lower ambient humidities in both SGA and AGA infants In full term and moderately pre-term SGA infants TEWL was lower than in corresponding AGA infants

KEY WORDS Water loss, water balance, small for gestational age infants

Infants with birth weights below 2 standard deviations (S D) for a given gestational age (24) are exposed to an increased risk of perinatal complications such as asphyxia, hypoglycaemia and meconium aspiration (23) Problems with postnatal respiratory and circulatory adjustment and temperature regulation are less pronounced in small for gestational age (SGA) infants than in pre term infants (23)

On physical examination SGA infants often look dry, with parched or peeling skin Contrary to appropriate for gestational age (AGA) infants, who usually lose up to 10% of their body weight during the first days of life due to a reduction in total body water (TBW) (3, 14), SGA infants have only a small decrease in body weight and sometimes even gain weight during the first days As body weight follows the hydration (11), this indicates that the water balance of SGA infants differs from that in AGA infants

The purpose of this study was to determine the amounts of water lost from the skin in SGA infants and to compare these with the values obtained earlier in AGA infants of different gestational ages (7, 8, 10)

SUBJECTS

The evaporation rate (ER, g/m²h) was measured on SGA infants, i e infants whose birth weights were 2 S D or more below the average for normal Swedish infants (12) Infants from multiple pregnancies, infants with chromosomal aberrations, known intrauterine infections or severe birth asphyxia and infants with meconium staining of the skin or excessive scaling were not included in the study One infant was excluded because of probable familial smallness

Measurements of ER were made at different ambient humidities on 14 infants (6 male, 8 female) born after 37 to 40 completed weeks of gestation (Table 1, series 1*a* and 1*b*), and on 10 infants (3 male, 7 female) born after 30 to 36 weeks (Table 1, series 1*b*) In 25 infants (10 male, 15 female) born after 30 to 40 weeks, measurements of ER were made and the transepidermal water loss (TEWL, g/m²h) was estimated (Table 1, series 2) When the measurements were started, the mean age of the infants was 11 1 h (range 1 6–22 3 h) All infants, except two (age 1 6 and 1 9 h) had received water and nutrients before the start of the measurements either orally as breast milk, or parenterally as 10% glucose solution

Previously published data from AGA infants (7, 10) were used for comparisons In series 1*a* data from 19

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Submitted Oct 5 1979
Accepted Nov 19, 1979

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METHODS

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allows accurate measurements and does not interfere with nursing routines The equipment for measurement of ER also gives data on the ambient relative humidity (RH_{amb}) and ambient vapour pressure ($P_{H_2O_{amb}}$) Most measurements were made with the prototype used in earlier studies and a few with commercially available equipment (Evaporimeter EP 1 ServoMed AB, Stockholm Sweden) The ambient air temperature (T_{amb}) skin temperature (T_{skin}) and body temperature (T_{body}) were measured with a YSI tele thermometer (43TA and 4002, using probes 405 421, 427 and 402, Yellow Springs Ohio USA) Recordings were made with a Watanabe recording system (Watanabe Instruments Corp , Tokyo Japan)

MEASUREMENT PROCEDURES

All measurements were made with the infant naked and placed in an incubator (AGA MK41 or MK241 AGA Medical Lidingo Sweden) The inflow of air to the incubator was kept at a constant level during the measurements in each infant and ranged between 8–15 l/min (cf 7) The ambient humidity was regulated as described previously (7) T_{skin} was recorded from the skin areas where ER measurements were made T_{body} was recorded as deep rectal temperature and T_{amb} was measured in the central part of the incubator By regulating T_{amb} T_{skin} and T_{body} were kept as constant as possible T_{body} was always between 36 0 and 37 0°C In infants with a gestational age of less than 35 completed weeks the system for servocontrol of skin temperature (AGA MK138 AGA Medical Lidingo Sweden) supplied with the incubator was used (cf 10) When the servocontrol was not employed the temperature in the incubator was set at 33 0–34 5°C The T_{skin} and T_{amb} values recorded during measurements in this study are given in Table 1

1 *Measurement of ER at different ambient humidities*

With the infant in the prone position intermittent measurements of ER were made on an interscapular skin surface while RH_{amb} was increased from 20% to 60% or decreased from 60% to 20% in steps of 5% through a change in the relation between dried and humidified air in the incubator at a constant air flow After each change in RH_{amb} steady state was reached in 5–10 min

2 *Estimation of transepidermal water loss*

With the infant in the lateral position and with RH_{amb} maintained at 50% ER was measured on the chest (*a*) on an interscapular skin area (*b*) and on a buttock (*c*) The transepidermal water loss (TEWL, g/m²h) i e. the cutaneous water loss per unit area, was calculated as described previously (7) using the expression

$$TEWL = 0\ 92\ \overline{ER}_{a,b,c} + 1\ 37 \quad (1)$$

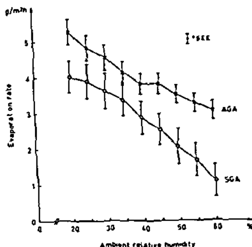


Fig 1 The relation between evaporation rate (ER) and ambient relative humidity (RH_{amb}) in full term small for gestational age infants (SGA) and full term appropriate for gestational age infants (AGA) S E E = standard error of the estimate

TREATMENT OF DATA

Data were obtained for ER, RH_{amb} $P_{H_2O_{amb}}$ T_{skin} T_{amb} and T_{body}

The arithmetic mean of the three skin temperatures measured for each TEWL was designated $\overline{T}_{skin\,a,b,c}$ (Table 1) All statistically significant differences reported in the following were obtained by testing with Student s *t* test

RESULTS

1a *Evaporation rate at different ambient humidities in full term small for gestational age infants compared with full term appropriate for gestational age infants*

When ER was measured from an interscapular skin area in the 14 full term SGA infants, higher values were obtained at a low RH_{amb} than at a high one (Fig 1, lower curve) This is consistent with the findings in full term AGA infants (Fig 1, upper curve, 7) and in pre term AGA infants (10) The values obtained in the full term SGA infants were lower than those in full term AGA infants at corresponding RH_{amb} For example, at an RH_{amb} of 50% the mean ER was 2.0±1 8 (S.D)

Table 1. *Infant data and mean skin and ambient temperatures in series 1 and 2*
Infants 1–29 are small for gestational age and infants 30–49 are appropriate for gestational age V=vaginal delivery CS=caesarean section

Infant	Gestational age (weeks)	Delivery	Weight at birth (kg)	Length at birth (m)	Measurement series	Series 1 T_{skin}±S D (°C)	Series 1 T_{amb}±S D (°C)	Series 2 $\bar{T}_{skin}$ (°C)	Series 2 $\bar{T}_{amb}$ (°C)
1	30	CS	0 840	0 330	1*b*, 2	36 1±0 1	34 9±0 5	35 9	34 4
2	31	CS	1 080	0 330	1*b*, 2	36 1±0 1	36 7±0 4	36 5	36 4
3	31	CS	1 175	0 375	1*b*, 2	36 1±0 0	37 2±0 5	36 2	36 3
4	33	CS	0 840	0 360	1*b*, 2	36 6±0 1	37 1±0 4	36 2	37 0
5	33	CS	1 700	0 410	1*b*, 2	36 1±0 2	37 1±1 0	36 5	36 1
6	35	CS	1 360	0 410	1*b*, 2	35 7±0 2	34 9±0 8	36 1	34 4
7	35	CS	1 970	0 450	2			36 5	35 7
8	36	CS	1 200	0 370	1*b*, 2	36 1±0 3	34 4±0 8	36 1	33 5
9	36	CS	1 900	0 460	1*b*	35 3±0 1	34 2±0 1		
10	36	CS	1 840	0 440	1*b*, 2	35 8±0 1	34 2±0 2	36 0	34 1
11	36	CS	1 950	0 450	1*b*, 2	35 8±0 1	35 4±0 2	35 5	36 6
12	37	V	1 790	0 430	1*a*, 1*b*, 2	36 1±0 2	35 0±0 7	36 3	34 7
13	37	CS	1 880	0 425	2			36 4	34 6
14	37	CS	1 900	0 440	2			35 9	32 5
15	37	V	1 930	0 440	1*a*, 1*b*	36 2±0 1	33 5±0 3		
16	37	CS	2 140	0 460	1*a*, 1*b*, 2	36 2±0 0	35 2±0 4	36 1	33 5
17	37	V	2 290	0 450	1*a*, 1*b*, 2	36 1±0 1	34 4±0 2	36 1	34 6
18	38	V	1 980	0 450	1*a*, 1*b*, 2	35 4±0 1	34 0±0 4	35 6	34 2
19	38	V	2 170	0 450	1*a*, 1*b*, 2	35 6±0 2	32 7±0 1	36 8	34 5
20	38	V	2 400	0 460	1*a*, 1*b*, 2	35 8±0 0	34 1±0 2	35 8	34 6
21	38	CS	2 450	0 455	1*a*, 1*b*, 2	35 9±0 1	35 5±0 6	36 1	35 0
22	39	CS	1 760	0 440	1*a*, 1*b*	36 2±0 2	34 3±0 3		
23	39	V	2 000	0 460	1*a*, 1*b*	35 6±0 2	34 2±0 1		
24	39	V	2 280	0 450	1*a* 1*b* 2	36 1±0 1	33 9±0 1	36 3	33 7
25	39	V	2 320	0 450	1*a*, 1*b*, 2	35 8±0 1	34 2±0 2	35 8	34 1
26	39	V	2 370	0 440	2			36 4	33 4
27	39	V	2 480	0 480	1*a* 1*b* 2	35 7±0 0	34 2±0 1	35 9	34 4
28	39	V	2 485	0 460	1*a*, 1*b*, 2	35 3±0 1	32 9±0 1	35 7	33 0
29	40	V	2 540	0 470	2			35 4	34 6
30	33	CS	2 180	0 440	1*b* 2	36 1±0 0	34 5±0 3	36 2	34 9
31	35	V	2 320	0 470	1*b* 2	36 0±0 1	35 1±0 2	35 9	35 0
32	35	V	2 400	0 440	1*b* 2	35 8±0 0	36 6±0 1	35 4	35 2
33	35	V	2 900	0 460	1*b* 2	35 6±0 1	33 5±0 2	35 6	33 9
34	36	V	2 670	0 460	1*b* 2	35 6±0 1	33 9±0 1	35 6	33 6
35	36	V	2 680	0 470	1*b* 2	35 6±0 1	33 8±0 1	35 5	33 7
36	36	CS	2 690	0 460	1*b* 2	35 1±0 1	32 1±0 1	35 8	32 8
37	36	V	2 880	0 490	1*b* 2	35 9±0 1	33 9±0 1	35 9	34 0
38	37	CS	3 100	0 490	2			35 9	33 9
39	37	CS	3 370	0 510	2			34 5	34 0
40	38	CS	3 140	0 490	2			35 7	32 7
41	38	CS	3 170	0 480	2			35 7	35 2
42	38	CS	3 410	0 510	2			35 8	33 7
43	38	CS	3 810	0 510	2			35 5	32 7
44	39	CS	2 700	0 470	2			35 9	33 1
45	39	CS	3 230	0 500	2			34 6	34 3
46	39	CS	3 340	0 510	2			36 2	34 5
47	39	CS	3 590	0 500	2			35 9	33 0
48	39	CS	3 700	0 530	2			34 9	34 3
49	39	CS	3 770	0 520	2			35 8	33 9

AGA infants (7) were employed Data from these 19 infants, 16 pre term AGA infants (10) and a further 8 pre term AGA infants (Table 1, infants 30–37) were used for comparisons in series 1*b* In series 2 data from 32 AGA infants (10) and previously unpublished data from 20 AGA infants (Table 1, infants 30–49) were used

The gestational age was estimated from pregnancy data and as described by Finnstrom (5) and also in most cases with the criteria proposed by Dubowitz et al (4) The ER measurements were made during the first 24 h after delivery The infants were often asleep during the measurements When awake they were quiet and calm and showed little spontaneous motor activity (cf 8) The respiratory and heart rates were normal for the age

fants were lower than in the AGA infants of the same gestational age In two infants negative values were found

The mean TEWL was 2 8±1 6 (S D) g/m²h for full term SGA infants (n=15, cf Table 1) and 5 3±1 4 (S D) g/m²h for full term AGA infants (n=22, cf Table 1 and Subjects) The difference in TEWL between these two groups of infants was statistically significant (p<0 001)

As regards the relation between TEWL and birth weight, the smallest SGA infants were found to have considerably lower TEWL values than AGA infants of the same weight (Fig 4) In SGA infants with a birth weight of 1±0 2 kg, TEWL ranged from 2 5 to 14 4 g/m²h, while in AGA infants with a corresponding weight it ranged from 27 8 to 75 0 g/m²h

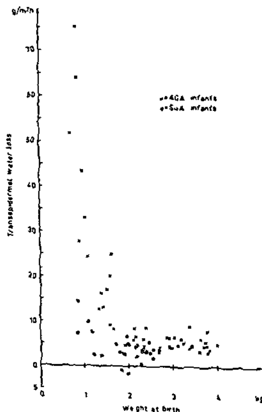


Fig 4 Transepidermal water loss (TEWL) in relation to birth weight in small for gestational age (SGA) infants and appropriate for gestational age (AGA) infants

DISCUSSION

Small for gestational age infants comprise a group of individuals who have one common characteristic, i e a low birth weight in relation to gestational age In view of the multifactorial aetiology of impaired intrauterine growth these infants cannot be considered as a homogeneous group In this study no infants with chromosomal aberrations or known intra uterine infections (6) were included Infants from multiple pregnancies also form a special subgroup which lies outside the frame of the present investigation Changes in the peripheral circulation which at the moment are difficult to quantitate, make infants with severe intrapartal or postnatal asphyxia of limited relevance to this study Thus the investigation was made on SGA infants with a confirmed gestational age, in a good general condition after birth, and with placental insufficiency as the only suspected or known cause of the disturbance of intrauterine growth

Both full term and moderately pre term SGA infants were found to have lower ER and TEWL values than AGA infants of the same gestational age The reaction to changes in ambient humidity was of the same magnitude in both groups of infants The difference in ER and TEWL found between full term AGA and full term SGA infants may be caused by many factors, both general, such as the metabolic rate (21), and hydration (3), and local for example the thickness and water content of the corneal layer of the epidermis (13, 18), the presence of vernix caseosa, the number of sweat glands and the peripheral blood flow The fact that general and local factors are interrelated, for instance the hydration of the skin influences the transepidermal water diffusion (22), makes it impossible to ascribe the differences in ER and TEWL to one or a few isolated factors

In the same way as AGA infants, pre term SGA infants have higher ER and TEWL values than full term SGA infants The differ-

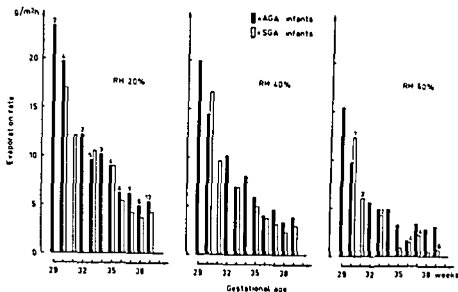


Fig 2 The relation between evaporation rate (ER) and gestational age at ambient relative humidities of 20, 40 and 60% in small for gestational age (SGA) infants and appropriate for gestational age (AGA) infants The numbers of AGA infants are given in the left part of the figure and those of SGA infants in the right part

g/m²h in the former and 3 5±1.1 (S D) g/m²h in the latter ($p<0\ 01$). There was a significant difference in the ER levels between the two groups of infants over the whole RH_{amb} range investigated ($p<0\ 001$, Student's *t*-test for parallel straight-line data)

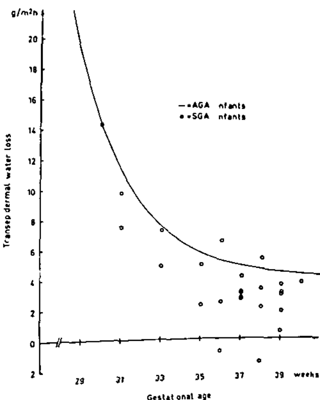


Fig 3 Transepidermal water loss (TEWL) in relation to gestational age in small for gestational age (SGA) infants and appropriate for gestational age (AGA) infants

1 b *Evaporation rate at different ambient humidities in small for gestational age infants at different gestational ages compared with appropriate for gestational age infants*

When ER was measured at different ambient humidities in SGA infants of varying gestational ages, the values were higher the lower the gestational age (Fig 2, open bars) This was true for all investigated levels of ambient humidity The ER values were higher when RH_{amb} was low than when it was high In Fig 2 (shaded bars) the ER values found in AGA infants (see Subjects) are given for comparison The ER values in SGA infants tended to be lower at all ambient humidities when the gestational age was more than 35 weeks The number of observations in each gestational week was too small to make statistical testing relevant

2 *Transepidermal water loss in small for gestational age infants compared with appropriate for gestational age infants*

TEWL was estimated in 25 SGA infants of varying gestational ages (Fig 3) In the figure the regression curve obtained from data on AGA infants (10) has been inserted As in the AGA infants, the TEWL values were higher with shorter gestational periods With a few exceptions, the TEWL values in the SGA in-

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Submitted Oct 1, 1979
Accepted Nov 14, 1979

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ence between AGA and SGA infants was less obvious at shorter gestational ages In intrauterine growth retardation the hydration of the foetus seems to be related to the severity of the weight reduction (2), but there are also reasons to believe that alterations in the epidermal layers are related to the duration and severity of the growth retardation

During the study some infants were found to have negative ER and TEWL values, i e they absorbed water from the environment Absorption of water by the skin in adults has been demonstrated by Buettner (1) The method used for measurements of ER in this study is valid also for absorption of water, although the vapour pressure gradient then lies in the opposite direction To explain the obtained negative ER and TEWL values further studies have to be made

In our earlier studies (7, 8, 10) it was found that TEWL depends not only on environmental factors, such as humidity and ambient temperature, but also on gestational age, body temperature and activity From the present results it is obvious that the nutritional status at birth also influences TEWL As seen in Fig 4, there were large variations in the TEWL values for the group of infants with birth weights around 1 kg with very high values for those with short gestations and low values for those that were most severely mal nourished

When supplying water to the newborn infant to replace losses from the skin calculation of the fluid needs in relation to weight thus seems inappropriate However, the losses from the skin cannot serve as a sole basis for recom mendations for fluid supply, as water lost with the respiration and stools as well as the renal function and nutritional needs also have to be taken into consideration

CONCLUSIONS

This study shows that during the first day of life

1 there is a linear relation between ambient humidity and evaporation rate in pre tern full term small for gestational age infants.

2 the evaporation rate from the sk lower in full term and moderately pre small for gestational age infants than in responding appropriate for gestational ag fants,

3 the susceptibility to changes in am humidity is not affected by the infant s propriateness for gestational age,

4 transepidermal water loss is lower in term and moderately pre term small for g tional age infants than in corresponding propriate for gestational age infants

ACKNOWLEDGEMENTS

This study was supported by grants from the Sw Medical Research Council (Project 19X-04998) Samaritan Foundation Stockholm the Forenade Mutual Group Life Insurance Company Stockholm the Medical Faculty of the University of Uppsala

of the University Hospital Uppsala is also grea acknowledged

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TRANSEPIDERMAL WATER LOSS IN NEWBORN INFANTS

V Evaporation from the Skin and Heat Exchange during the First Hours of Life

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ABSTRACT Hammarlund, K., Nilsson, G. E., Öberg, P. Å. and Sedin, G. (Department of Paediatrics, University Hospital, Uppsala, and Department of Biomedical Engineering, Linköping University, Linköping, Sweden). Transepidermal water loss in newborn infants. V. Evaporation from the skin and heat exchange during the first hours of life. Acta Paediatr Scand, 69.385, 1980.—The amount of water evaporated from the skin was studied in 10 healthy newborn infants from their first minute of life, while being taken care of in the delivery room, and in 11 infants treated in incubators from their 30th min of life. The heat lost by evaporation, radiation and convection was calculated. Evaporation from the skin was very high during the first minutes after birth and was the main cause of heat loss during the first 15–30 min of life Thereafter the amount of heat lost depended on the conditions under which the infant was nursed Higher convective and radiative heat losses were found in delivery rooms than in incubators.

KEY WORDS Water loss, water balance, newborn infants, temperature regulation

After birth the human infant is subjected to a period of cold stress with a resulting decrease in body temperature This decrease is partly physiological, as the body temperature at birth is higher than that in subsequent life Exposure to a cooling environment may also play a role in stimulating the onset of breathing (4), but early heating does not impede or delay this onset (3)

During the first 15 min of life the oxygen uptake is high, oxygenation is improved and the acid-base status is normalized (25) After the age of 0 5 h an increase in metabolic rate has been observed in response to cold stress (5), but it has not been established with certainty to what extent the infant can increase his heat production during the first 10–15 min of life (17, 24) Later the well-oxygenated infant can triple his heat production on exposure to cold stress, but even a heat production of this magnitude would be insufficient to meet the cold stress soon after birth (19)

Heat loss by evaporation of amniotic fluid from the skin surface has been considered one of the major causes of cooling of the infant. Both for evaporation and for other modes of heat loss environmental factors are of great importance and at the same time difficult to control.

Direct measurements of the evaporation of amniotic fluid and transepidermal water loss are now possible by means of a method based on determination of the vapour pressure gradient close to the skin surface (7, 14, 15, 16)

The aim of this study was to investigate the amount of water evaporated from the skin of newborn infants during the first hours of life and to estimate the heat exchange in two common clinical situations, namely during care in delivery rooms and in incubators.

SUBJECTS

The evaporation rate (ER, g/m²h) was measured on 21 healthy newborn infants, born after 37 to 41 completed weeks of gestation (Table 1) The birth weights of all in-

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SUBJECTS

The evaporation rate (ER, g/m²h) was measured on 21 healthy newborn infants, born after 37 to 41 completed weeks of gestation (Table 1) The birth weights of all in

Table 2 *Mean skin, body and ambient temperatures, ambient humidity and ambient vapour pressure in series I*

Age (min)	T_{skin}±S D (°C)	T_{body}±S D (°C)	T_{amb}±S D (°C)	RH_{amb}±S D (%)	$P_{H_2O\,amb}$±S D (kPa)
1	34 1±1 8	37 3±0 9	25 8±1 4	42± 9	1 6±0 4
5	33 7±1 6	37 0±0 9	25 8±1 4	46± 9	1 8±0 5
15	34 5±1 1	36 6±0 9	25 9±1 4	45±10	1 8±0 4
30	35 1±1 1	36 4±0 8	25 9±1 4	42± 9	1 7±0 5
60	34 4±1 0	36 2±0 5	25 8±1 6	39± 9	1 5±0 4

f the delivery room were measured during the period under study and the mean value was calculated (T_{wall_r})

Series 2

After suctioning and a brief examination, the 11 infants born by Caesarean section were covered with a dry towel and placed in a transport incubator (Drager 5100, Dragerwerk Lubeck GFR)

[illegible]

50% and a T_{amb} that was kept slightly above the thermoneutral range (12) The infants were naked and placed in the lateral position During measurements they were mostly asleep and showed little motor activity

Measurements of ER were made from the chest, an interscapular skin area and a buttock T_{skin} was recorded from each skin area at the time of each measurement *Simultaneously T_{body} and T_{amb} were recorded (Table 3)* Measurements from the three skin areas were repeated at 10–20 min intervals until at least 2 h of life

TREATMENT OF DATA

Data were thus obtained for ER, RH_{amb} $P_{H_2O\,amb}$, T_{skin}, T_{amb} T_{body}, T_{wall_i} and T_{wall_r}

The evaporative water loss from the skin (EWL_{skin}, g/m²h) was estimated from the equation presented by Hammarlund et al (7) for estimation of transepidermal water loss (TEWL, g/m²h), using ER data from the chest (*a*), an interscapular skin area (*b*), and a buttock (*c*)

$$TEWL = 0\ 92\ \overline{ER}_{a,b,c} + 1\ 37 \qquad (1)$$

In series 1 the skin was covered with amniotic fluid when measurements were made 1 and 5 min after birth.

[illegible] considered [illegible] surfaces EWL_{skin} at 0 25, 0 5 and 1 h *in series 1* and at all ages in series 2 was calculated from the equation for *TEWL even though the evaporative water loss at the* lower ages partly consisted in evaporation of amniotic fluid and partly in transepidermal water loss

In series 2 linear interpolation was used when the time interval between two sets of ER measurements $\overline{ER}_{a,b,c}$ deviated from 15 min

The arithmetic mean of the three measured skin temperatures was designated $\overline{T}_{skin\,a,b,c}$ (Tables 2 and 3) In series 1 the $\overline{T}_{skin\,a,b,c}$ values at 1 and 5 min are the temperatures of the skin areas on which the ER measurements were made $\overline{T}_{wall_r}$ was calculated as the arithmetic *mean of the measured wall temperatures in the delivery* room A separate study showed that $\overline{T}_{wall_i}$ was related to the incubator air temperature by

$$\overline{T}_{wall_i} = 0\ 775\ T_{amb} + 5\ 20 \qquad (2)$$

in the T_{amb} interval 33–37°C

Table 3 *Mean skin, body and ambient temperatures and ambient vapour pressure in series 2*
The ambient humidity was kept at 50%

Age (h)	T_{skin}±S D (°C)	T_{body}±S D (°C)	T_{amb}±S D (°C)	$P_{H_2O\,amb}$±S D (kPa)
0 50	34 8±0 5	36 3±0 3	35 5±0 8	2 9±0 13
0 75	35 2±0 4	36 1±0 3	35 6±0 5	2 9±0 10
1 00	35 4±0 4	36 2±0 4	35 9±0 7	2 9±0 12
1 25	35 6±0 4	36 3±0 4	35 9±0 7	2 9±0 10
1 50	35 7±0 4	36 4±0 4	35 9±0 7	2 9±0 10
1 75	35 9±0 4	36 5±0 4	35 9±0 5	2 9±0 05
2 00	35 9±0 4	36 6±0 4	35 8±0 5	3 0±0 07

Table 1. *Infant data in series 1 and 2*
V = Vaginal delivery, CS = Caesarean section

Infant	Gestational age (w)	Delivery	Weight at birth (kg)	Length at birth (m)	Sex	Measurement series
1	39	V	3 270	0 500	M	1
2	39	V	3 420	0 500	M	1
3	39	V	3 390	0 500	M	1
4	39	V	3 550	0 500	M	1
5	38	V	3 360	0 510	M	1
6	41	V	4 120	0 500	M	1
7	40	V	3 400	0 510	M	1
8	39	V	3 530	0 510	F	1
9	38	V	3 630	0 510	M	1
10	41	V	4 755	0 560	M	1
11	38	CS	3 400	0 505	F	2
12	39	CS	3 120	0 480	F	2
13	38	CS	3 860	0 535	M	2
14	38	CS	3 670	0 505	M	2
15	39	CS	2 650	0 480	M	2
16	38	CS	3 790	0 510	F	2
17	38	CS	3 000	0 470	F	2
18	37	CS	3 190	0 500	M	2
19	39	CS	3 260	0 500	F	2
20	38	CS	4 200	0 520	F	2
21	39	CS	3 810	0 520	M	2

fants were appropriate for their gestational age (13) The infant s skin was not wiped before measurements In 10 infants born by vaginal delivery (series 1) measurements were made in the delivery room starting at the first minute of life, and in 11 infants born by Caesarean section (series 2), measurements were made with the infant in an incubator The latter measurements were started at approximately 0 5 h of age The ambient temperature in the incubator was kept slightly above the thermoneutral range (12)

METHODS

ER was measured by a method based on determination of the vapour pressure gradient in the air layer close to the skin surface (14 15 16) *The method which we have* used since 1974 in studies on water loss in full term infants (7, 8, 11, 16, 20 21) *and in pre term infants (9 10)* allows repeated measurements at short intervals The equipment [illegible] ambient h[illegible] ($P_{H_2O\,amb}$) [illegible] perature (T_{skin}) body temperature (T_{body}) and incubator *wall temperatures* (T_{wall_i}) were measured with a YSI telethermometer (43 TA, using probes 405, 421 427 and 402 Yellow Springs, Ohio, USA) With a specially designed silent electronic switch, *temperatures* from six different sources could be recorded every minute The wall temperatures in the delivery room (T_{wall_r}) were measured with alcohol thermometers All recordings were made with a Watanabe recording system (Watanabe Instruments *Corp* Tokyo Japan)

MEASUREMENT PROCEDURE

Series 1

In the 10 infants born by vaginal delivery measurements of *ER* were made *approximately 1 5, 15, 30 and 60 min* after birth As soon as the infant was delivered (1 min) ER was measured from an accessible skin area on the chest or back

The infant lay on the delivery table at a distance of 0 3–0 6 m from the mother s buttocks and legs The infant s skin was not washed or wiped before measurements and care was taken to avoid measurements from skin surfaces *soiled with blood or mucus*

When the first ER measurement was completed the infant was placed on its side on a towel on the mother s *chest and 5 min after birth ER was again measured* from the same skin area as before

At 0 25 0 5 and 1 h of age ER was measured from the chest an interscapular skin area and a buttock When the 0 25 h measurements were completed the infant was covered with a dry towel and left on the mother s chest until the age of 0 5 h when the covering towel was removed and measurements were repeated

The infant was then taken from the delivery room for weighing and measuring and was clothed and placed underneath a radiant heater for about 0 25 h before being returned to the delivery room for the 1 h measurements While these measurements were being made the infant lay naked on its side in a shallow cot placed alongside the delivery table

T_{skin}, T_{body} and T_{amb} were recorded with each ER measurement (Table 2) The temperatures of the walls and floor

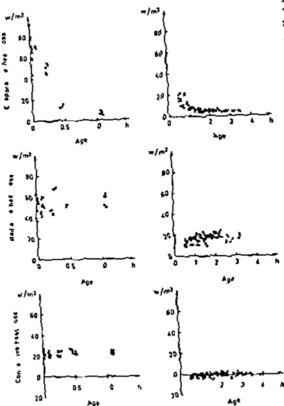


Fig 2 Heat exchange in relation to age in series 1 (left) and series 2 (right)

Exchange of heat

Evaporation As $H_{e\ ap}$ is directly proportional to the amount of water evaporated this heat loss was high directly after birth when the in fant was covered with amniotic fluid and gradually decreased during the first hours of life (Fig 2 upper part)

Radiation The infants lost heat to the en vironment through radiation both in delivery rooms and in incubators (Fig 2 middle part) The mean temperature of the walls facing the infants was 25 3±2 3 (S D) °C in the delivery rooms and 33 0±0 5 (S D) °C in the incuba tors which gave a H_{rad} in the delivery rooms that was more than three times that in the incubators The changes in H_{rad} with age were small in both measurement series

Convection In series 1 the infants were ex posed to a mean ambient temperature of about 26°C (Table 2) and H_{conv} was calculated to be about 25 W/m² (Fig 2, lower left) There were no obvious changes with age In series 2, where the mean ambient temperature in the incubator was 35 8°C (Table 3), H_{conv} was close to zero, and several infants actually gained heat through convection (Fig 2 lower right)

Body temperature

The body temperature of the infants in both series decreased with age during the first 0 5 h after birth (Fig 3), although care was taken to reduce excessive heat loss (see measurement procedure) Between the ages of 0 5 and 1 h T_{body} was at approximately the same level in the two series (cf Tables 2 and 3) In series 2 there was an increase in T_{body} after 1 h of life

DISCUSSION

The heat exchange between the infant and the environment immediately after birth is influ enced by a multitude of biological and physical factors This makes it necessary to keep many physical variables controlled or monitored during measurements and to limit the validity of the obtained results to particular clinical situations and environmental conditions, such as those in the present investigation

In earlier studies on evaporation from the skin (7, 8 10 11) we have noticed differences in the heat lost by evaporation from the skin surface of newborn infants in relation to en vironmental factors activity and gestational age This has focused our interest on heat ex change and the present study has therefore dealt with two common clinical situations in which the knowledge of heat exchange is limited During the measurements the environ mental factors were kept as constant as pos sible The rate of evaporation and the tem peratures have been used to calculate the heat exchange due to evaporation, radiation and convection, using expressions which after ap proximations are reasonably valid for the clin ical situations in question

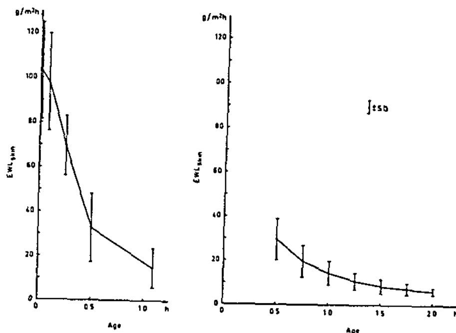


Fig. 1. Evaporative water loss in relation to age in series 1 (left) and series 2 (right). S.D.=standard deviation.

Calculation of heat exchange

Heat exchange between the skin and the environment occurs through evaporation, conduction, radiation and convection (for review see 2). The calculations in this study are based on data for evaporative water loss and skin, ambient and wall temperatures.

Evaporation. The heat lost by evaporation from the skin per unit area and unit time (H_{evap} (W/m²)) can be expressed as

$$H_{evap} = k_1 \cdot EWL_{skin} \cdot [3.6\times10^3]^{-1} \qquad (3)$$

where k_1 is the latent heat of evaporation of water (2.4×10³J/g), EWL_{skin} is the evaporation from the skin per unit area and hour (g/m²h) and 3.6×10³ is the correction factor for time (s).

Conduction. The conductive exchange of heat depends on the temperature of the infant's skin, the temperature of the materials in direct contact with the skin and the thermal properties of these materials. As the infant was placed either on the mother's chest or on a thick mattress in a warm incubator, the heat exchange through conduction could be considered to be small and was therefore omitted from the further calculations in this study.

Radiation. The emission of radiation from the body and the surfaces of the environment leads to an exchange of heat, the rate of which is determined by the emissivity (e_1~1) and the mean temperature ($\bar{T}_1$ (K)) of the skin and the emissivity (e_2~0.97) and the mean temperature ($\bar{T}_3$ (K)) of the surrounding walls. Radiative heat loss or H_{rad} (W/m²) can be expressed as

$$H_{rad} = S_0 \cdot e_1 \cdot e_2 \cdot (\bar{T}_1^4 - \bar{T}_3^4) \qquad (4)$$

where S_0 is Stefan Boltzman's constant (5.7×10⁻⁸ W/m²K⁴).

In series 1 T_3 is the mean temperature of the walls in the delivery room and in series 2 it is the mean temperature of the incubator walls.

Convection. Convective heat exchange between the skin and the environment occurs as free convection or forced convection. For adults free convection predominates at air velocities below 0.27 m/s (2). This value may be somewhat lower for smaller individuals. The average air velocity in the incubator was below 0.15 m/s and the air velocity in the delivery room was even lower. The contribution from forced convection was therefore not included in this study and the heat exchange per unit time through convection (H_{conv} (W/m²)) was calculated as

$$H_{conv} = k_3 \cdot (\bar{T}_1 - \bar{T}_4) \qquad (5)$$

where k_3 is the convection coefficient (2.7 W/m²K (1)), $\bar{T}_1$ (K) is the mean skin temperature and $\bar{T}_4$ (K) is the mean temperature of the ambient air either in the delivery room (Series 1) or in the incubator (Series 2).

RESULTS

Evaporation of water

In series 1 the evaporation from the skin surface during the first minute of life was high, with an EWL_{skin} of 103±22 (S.D.) g/m²h. EWL_{skin} was markedly decreased to 32.7±15.5 (S.D.) g/m²h at the age of 0.5 h and had reached a more moderate level at the age of 1 h (Fig. 1). At 0.5 h EWL_{skin} in series 2 was 29.7±9.6 (S.D.) g/m²h, gradually decreasing to 6.3±1.5 (S.D.) g/m²h by the age of 2 h.

large amount of heat is lost, especially by evaporation and radiation, during the first 0 5h after birth When the condition of the infant makes it impossible to reduce the heat loss by covering it with warm towels, for example, heat should be supplied and this can be done with a radiant heater or an incubator

In healthy infants the fall in body temperature after birth (Fig 3) can be kept at a moderate level even when the infant is nursed in its mother s arms, *provided that* adequate precautions are taken to prevent excessive heat loss immediately after delivery

CONCLUSIONS

Under the conditions in which this study was performed it can be concluded that

1 the evaporation of water and the evaporative heat loss from the skin of newborn infants are very high during the first minutes of life and decrease gradually to moderate levels during the first hour,

2 the heat lost through radiation is three times higher in the delivery room than in the incubator,

3 the heat exchange through convection is about 25 W/m² in the delivery room and close to zero in the incubator

ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Medical Research Council (Project 19X-04998) the Samaritan Foundation Stockholm the Forenade Liv Mutual Group Life Insurance Company Stockholm and the Medical Faculty of the University of Uppsala. The authors wish to thank B Ostmark S G Nilsson S G Norberg and H Pettersson for skilful laboratory and technical assistance The valuable help of the staff of the Neonatal Unit and the Obstetric Unit of the University Hospital Uppsala is also gratefully acknowledged

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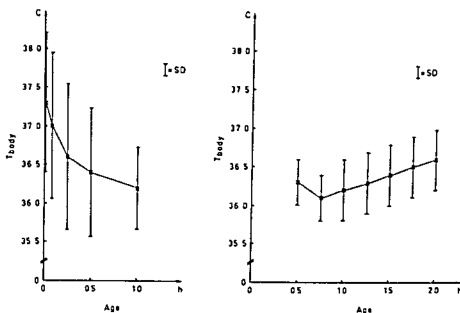


Fig. 3. Body temperature in relation to age in series 1 (left) and series 2 (right). S.D. = standard deviation.

At birth the infant is covered with amniotic fluid and it seems probable that the distribution of the fluid is almost uniform over the body surface. When 1 g of water evaporates from the skin surface 2.4×10^3 J are lost (26). Loss of water from the skin surface by wiping or through absorption to the bedding or to a covering towel causes a significantly lower heat loss (1.3×10^3 J/g). The very high evaporative heat loss found soon after birth (Fig. 1) implies that measures to prevent excessive evaporation should be taken as soon as possible.

The fact that the evaporative water loss was at about the same level 1 h after birth in the two series (Fig. 1) even though the ambient humidity and ambient P_{H_2O} were lower in series 1 may have been due to absorption of water by the covering towel used in this series. The evaporation of amniotic fluid accounts for the high values obtained soon after birth, while the lower values in the later part of the studied period mainly derive from transepidermal water loss.

The extent of the body surface area that exchanges heat with the environment is dependent on the type of heat exchange (22), on the position and geometry of the body (2, 6) and on the size and frequency of body movements (23). When skin surfaces are close to each other, the heat exchange due to evaporation, radiation and convection is diminished or abolished. For example, surfaces with the same temperature facing each other do not lose heat by radiation (2, 6), and adults assuming the foetal position thus reduce their radiating surface area by around 40% (6).

In this study all determinations of heat exchange were made per unit area of body surface losing heat, that is of the surfaces exposed to the ambient air or facing the walls of the delivery room or the incubator. The accuracy of the calculated heat exchange depends both on the accuracy of the measurements of temperatures and ER and on the validity of the approximations made. While it is not possible to state the absolute amount of heat exchanged between the infant and the environment, the results allow comparisons of the relative contributions of the different kinds of heat exchange in the two clinical situations studied.

The threefold difference in radiative heat loss between series 1 and 2 was caused by the difference in the temperatures of the walls of the delivery rooms and incubators. Differences in wall temperatures in series 1 also caused a 25% higher radiative heat loss in five infants studied during the cold season than in five studied during the warm season.

From this investigation it is obvious that a

Acta Pædiatr Scand 69: 393–396, 1980

STUDY OF HEART RATE VARIABILITY IN SICK NEWBORN INFANTS

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ABSTRACT. Jenkins, J. G., Reid, M. McC. and McClure, B. G. (Special Care Baby Unit, Royal Maternity Hospital, and Department of Child Health, Queen's University, Belfast, N. Ireland). Study of heart rate variability in sick newborn infants. Acta Paediatr Scand, 69, 393, 1980.—Heart rate variability has been studied in a group of 66 newborn infants for periods of up to 72 hours from birth Long term variability was reduced in infants suffering from the idiopathic respiratory distress syndrome and this was more marked with severe respiratory distress requiring mechanical ventilation. Persistent reduction in long term variability was associated with increased mortality. Reduction in heart rate variability may be due to high levels of sympathetic activity.

KEY WORDS. Heart rate variability, idiopathic respiratory distress syndrome

In the normal fetus and newborn the heart rate varies as a result of the balance between sympathetic and parasympathetic impulses to the sinoatrial node originating mainly in the cardioregulatory centres in the medulla under the modifying influences of the higher centres (14)

Continuous monitoring of the fetal heart rate is a useful method of assessment of fetal well being in the antenatal and intrapartum periods (13) The instantaneous heart rate in the normal fetus is seen to be varying and certain heart rate patterns have been recognised as indicating fetal distress These include decelerations associated with and extending beyond uterine contractions (Type II dips), and loss of the normal heart rate variability (3)

Recent studies (9, 10) have suggested that ill newborn infants may also lose their heart rate variability In this paper we present our experience of heart rate monitoring in newborn infants with and without the idiopathic respiratory distress syndrome (IRDS).

PATIENTS AND METHODS

During the period 1st March 1977 to 31st August 1978 babies admitted to the Special Care Baby Unit in the Royal Maternity Hospital, Belfast, were monitored using Corometrics 512 cardiorespirographs (CRG) The heart rate was monitored continuously from as soon as possible after admission until age 72 hours or prior death In the study group of 66 infants there were 41 males and 25 females Their mean birth weight was 1940 g Twenty-three infants weighed less than 1 5 kg, a further 28 less than 2 5 kg, and the remaining 15 infants weighed between 2 5 and 4 1 kg Eleven infants were of 28 weeks gestation or less, a further 24 were between 29 and 32 weeks, 20 between 33 and 36 weeks and remaining 11 were between 37 and 42 weeks Their mean gestational age was 32 9 weeks Seventeen infants died and 49 survived the neonatal period

Patients were classified into 3 groups

Group 1 (29 patients) These infants did not suffer from IRDS but were admitted because of extreme low birthweight or some non respiratory illness

Group 2 (23 patients) These infants had mild to moderate IRDS requiring either oxygen therapy alone or continuous positive airway pressure (CPAP) if the $P_{a_{O_2}}$ fell below 60 mmHg (8 0 kPa) in an F_iO_2 of 0 6

Group 3 (14 patients) These infants had severe IRDS requiring intermittent positive pressure ventilation because of failure to maintain a $P_{a_{O_2}}$ of greater than 50 mmHg (6 7 kPa) in an F_iO_2 of 1 0, on continuous positive airway pressure, or because of recurrent apnoeic attacks

The diagnosis of IRDS was made clinically by one of us

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Submitted Sept 21, 1979
Accepted Nov 14, 1979

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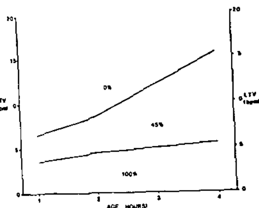


Fig. 3. Prediction of incidence of IRDS from LTV during first 4 hours of life

rising to 6 at 4 hours, all infants developed IRDS. Forty-five per cent of infants with intermediate levels of LTV during the first 4 hours of life developed IRDS.

Persistent reduction in LTV was associated with a poor prognosis for those infants (Fig. 4). LTV greater than 10 after age 8 hours was associated with an excellent prognosis whereas mortality was 40% if LTV remained less than 8 at age 48 hours, and rose to 60% if LTV had not improved further by age 72 hours. This is in agreement with the results of Rudolph et al. (9).

Using a method of heart rate analysis similar to that used in this study, Cabal et al. (1) found similar results. Kero, using a different method of variability analysis, noted a similar loss of variability in infants with IRDS and infants who died in the neonatal period (5). He reported that during the first, second and fourth days of life, STV discriminated poorly between normal infants and those with IRDS, and that there was little difference in values of STV between infants with differing degrees of severity of IRDS. This is in keeping with our finding that STV did not correlate well with either severity of IRDS or outcome. However, Kero also reported that a different measure of beat-to-beat variability (RMSSD) did give useful discrimination between these groups after the first day of life.

The mechanisms by which LTV is reduced are not easily explained, as the normal control of heart rate is complex. Under normal circumstances there would appear to be oscillation in parasympathetic and sympathetic impulses arising in the medulla and passing to the sinoatrial node. The cardioregulatory centre is in turn under the control of higher central nervous system centres and is influenced by information obtained at central and peripheral levels (11). Alteration in blood oxygen (4) and carbon dioxide (8) tensions as well as changes in pH (7) have been shown to affect variability. We are currently investigating these relationships further.

Two possible mechanisms have been postulated to account for the reduction in heart rate variability seen in IRDS. First, it is known that circulating catecholamine levels are higher in infants with IRDS than in normal infants (2). This may be indicative of a situation of severe stress. The rise in baseline heart rate and loss of variability seen in IRDS may be the result of an abnormally high level of sympathetic activity.

Second, it is known that both infants and adults with severe brain damage show marked loss of heart rate variability (6, 12), and this may be due to loss of the influence of higher cerebral centres on the cardioregulatory centres (5, 10). In IRDS this may result from anoxia or other metabolic abnormality. However, in this study, a number of infants who showed marked loss of LTV survived and



Fig. 4. Prediction of mortality from LTV at ages 8 to 72 hours.

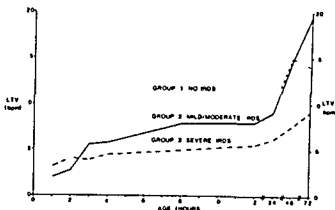


Fig 1 Differences in LTV between IRDS groups

(GMcC or MR) and confirmed by a chest radiograph in all cases

The CRG is attached to the infant using three electrodes, one on either side of the chest at the position of maximal respiratory movement, with a third electrode attached to the right thigh The signals are preamplified and filtered to obtain the ECG and transthoracic impedance

The R wave of the ECG is used to calculate the R-R interval *in milliseconds* This is converted to the instantaneous heart rate expressed in beats per minute which is recorded on paper moving at 1 or 3 cm per minute

Heart rate variability is also calculated Long term variability (LTV) is defined in this study as the difference between maximal and minimal instantaneous heart rates over a period of 512 cardiac cycles, expressed in beats per minute Short term variability (STV) is defined as the average of the beat to beat interval differences over 512 cycles, expressed in milliseconds These values are then displayed as columns on the paper trace Analysis of the heart rate traces was carried out by one of us (J J) with out knowledge of the clinical condition of the infants Periods during which there was marked deviation of heart rate from the baseline, for example during apnoea induced bradycardia were excluded from the analysis

RESULTS

LTV was analysed at ages ranged from 1–72 hours The mean values for each group at given times are shown in Fig 1 Infants in group 1 had LTV ranging from 10 to 15 beats per minute during the study period In both IRDS groups there was initial loss of LTV but by age 8 hours this had begun to improve in the group 2 infants and by 24 hours it was similar to those in group 1 However LTV of infants in group 3 remained low throughout the study period

LTV for each infant was also compared with outcome and the mean values for survivors and non-survivors are shown in Fig 2 The mean values of LTV for infants who died wer consistently lower than for those who survived

As the values of LTV were not normally distributed in this sample, statistical analysis was performed using the Mann-Whitney U test This showed statistically significant differences ($p<0.05$) between infants with no IRDS and those with (i) mild/moderate IRDS at ages 1–4 hours, and (ii) severe IRDS at ages 1–12 hours Differences between the mild, moderate and severe groups were significant at 48 and 72 hours There were also significant differences between survivors and those infants who died at 24, 48 and 72 hours

STV was analysed in a similar fashion but did not show significant associations with either severity of IRDS or outcome

DISCUSSION

Infants with IRDS showed less long term heart rate variability than those infants who did not have IRDS and this loss of LTV was more marked in infants with severe IRDS requiring mechanical ventilation It was possible to predict from knowledge of an infant s LTV during the first four hours of life whether it was likely to develop IRDS (Fig 3) If LTV was greater than 6 at 1 hour, rising to 15 at 4 hours, no infant developed IRDS Conversely, if LTV was less than 4 at 1 hour,

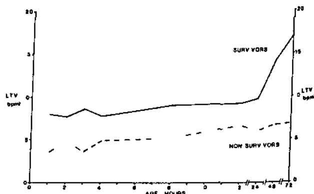


Fig 2 Differences in LTV between survivors and non survivors

Acta Pædiatr Scand 69 397–401, 1980

INACTIVATED POLIOVACCINE ADVERSE REACTIONS AND ANTIBODY RESPONSES

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ABSTRACT Ruuskanen, O , Salmi, T T , Stenvik, M and Lapinleimu, K (Department of Paediatrics University of Turku, and the Central Public Health Laboratory, Helsinki, Finland) Inactivated poliovaccine adverse reactions and antibody responses Acta Paediatr Scand, 69 397, 1980.—Adverse reactions and antibody responses after inactivated poliovaccine were studied in 380 children Fever reaction (≥37 5°C rectally) was recorded in 14% of children after the first poliovirus vaccination and in 19% after the second Restlessness was [illegible] 11% of the children Fever reaction exceeding 38 5°C was seen in 5% of [illegible] responses were lo[illegible] respectively After[illegible] titers were 1 67, [illegible] type 2 in 2%, and to type 3 in 10% Only one child was triplenegative All seronegative children were revaccinated and in all vaccinees a seroconversion ocurred two weeks after the booster dose The combination of inactivated poliovaccine to DPT (Diphtheria, Tetanus, Pertussis) vaccine induced no significant change in poliovirus antibodies or in adverse reactions

KEY WORDS Inactivated poliovaccine, poliovirus antibodies, adverse reactions

There are two types of poliovaccines available Live oral vaccine is used in most countries whereas Finland, Holland, Ireland, Sweden and two provinces in Canada are the only countries relying solely on inactivated vaccine The constant question which type of vaccine should be used has recently been repeatedly discussed (4–7) Both vaccines have shown to be highly effective With live oral vaccine it is easy to vaccinate a great part of the population in a short time, but it may on rare occasions cause paralytic disease in recipients of the vaccinee or their contacts On the other hand, parenterally administered inactivated poliovaccine induces no severe side effects, but 80–90% of the population should be completely vaccinated to achieve high herd immunity This seems to be possible only in a few countries (12, 13, 16)

This study reports adverse reactions and antibody responses of inactivated poliovaccine Furthermore, the effect of the combination of poliovaccine with DTP vaccine on antibody response was studied

MATERIALS AND METHODS

The material consists of two groups of 190 children who were vaccinated according to the two immunizing schedules used in Finland (Table 1) At the age of five months group A of the children received III DTP vaccine and group B received III DTP I poliovaccine Group A got the I and II poliovaccines at the age of six and seven months and group B the second poliovaccine at the age of six months At the age of 24 months all children received IV DTP III poliovaccine

Commercially licenced DTP DTP polio and poliovaccines from the same lot were used throughout the study except at the age of 24 months DTP poliovaccine (Orion Diagnostica Helsinki Finland) contained 80 units of [illegible]eria toxoid 80 units of tetanus toxoid and 8 units of

show no evidence of brain damage at follow up We feel that this finding lends support to the first hypothesis.

During this study it has become apparent that poor electrode contact and electrical inference may disturb the recording of LTV. A more significant source of error is the occurrence of cardiac decelerations with, for examples, apnoeic attacks, leading to an apparent increase in LTV.

The results of this study suggest that a reduction in long term variability is a consistent finding in severe idiopathic respiratory distress syndrome, and, if persistent, usually indicates poor prognosis.

ACKNOWLEDGEMENTS

We would like to thank Professor I J Carré and Dr W A B Campbell for permission to study infants under their care, the nursing and medical staff of the Royal Maternity Hospital Nursery for their help, and Lynda Thompson for typing the manuscript We are also grateful to the Lagan Valley Ladies Circle for financial assistance

Dr Jenkins is in receipt of a Department of Health and Social Services Research Fellowship

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Submitted May 5 1979
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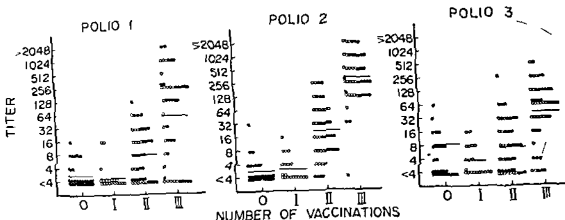


Fig 2 The antibody titers to poliovirus type 1 2 and 3 before vaccinat on and after the first second and third vaccination with inactivated poliovaccine ●=Group A (The children received poliovaccines at the age of 6 and 7

tion of the vaccination No differences in the amount and nature of adverse reactions were found between the first and second poliovirus vaccinations and between different sexes At six months of age before polio vaccination 29% of the children had antibodies against poliovirus types 1 and 2 (titer ≥1 4) and 43% against type 3 derived from the mother (Fig 2) The first poliovaccine induced no significant changes in antibody titers One to two months after the second vaccination, at the age of 8 months antibody responses were low the geometric mean antibody titers for types 1 2 and 3 being 1 8 1 22 and 1 8, respectively 25% of vaccinees remained seronegative for type 1 9% for type 2, and 35% for type 3 After IV DTP III polio vaccination at the age of two years the geometric mean teters of poliovirus antibodies were 1 67, 1 335 and 1 48 No measurable antibodies were found to poliovirus type 1 in 23%, to type 2 in 2% and to type 3 in 10% Only one of the children was triplenegative, one double (to types 1 and 2) and 12 single negative (seven to type 1 and five to type 3) There were no significant differences in the antibody responses between groups A and B of children with different immunization schedules (Table 3)

DISCUSSION

After vaccination with inactivated poliovaccine fever reaction and restlessness were the most common reactions Clinically significant fever reaction (≥38 5°C) was recorded in only 5% of the vaccinees Fever started in most cases on the day of the vaccination and lasted 1–2 days We feel that the fever reactions in few children starting 6 days or later after the

Table 2 Occurrence (%) of adverse reactions after inactivated poliovaccine

Reaction	First vaccination $N=119$ (63%)*	Second vaccination $N=106$ (56%)*
Fever		
≥37 5°C	14	19
≥38 5°C	5	5
Restlessness	15	14
Local reaction	4	7
Vomiting	7	5
Diarrhea	5	4
Other reactions	2	1
Contact to nurse or physician	1	1
Drug treatment	4	5
Similar symptoms in unvaccinated siblings or playmate	5	5

* Percentage of the recovery of the questionnaires

Table 1 *The vaccination program and serum collections*

Age (months)	Group A 190 children	Group B 190 children	48 children
3	I DTP	I DTP	
4	II DTP	II DTP	
5	III DTP	III DTP I polio	
6	I polio	II polio	Serum sample
7	II polio	–	
8	–	–	Serum sample
24	IV DTP III polio	IV DTP III polio	
25	–	–	Serum sample

pertussis bacteria per ml combined in the equal volume with inactivated poliovaccine (Inactivated Poliovaccine, R I T , Genval, Belgium) which contains poliovirus type 1 (strain Charleston), type 2 (strain MEF 1) and type 3 (strain Saukett) The virus strains were inactivated with formalin Before inactivation each single dose of 0 5 ml had titers at least 10^7 $TCID_{50}$ for each type The same poliovaccine was used in separate poliovirus immunizations The vaccines were adsorbed with aluminum phosphate

Data concerning adverse reactions after the vaccinations were collected using questionnaires given to the parents of the children at the time of poliovirus vaccinations at the age of six months Thus reactions to the first and second poliovirus vaccinations were monitored The questionnaires were returned by post 2–3 weeks later

Serum samples were collected from 48 children at the age of six, eight and 25 months for determination of antibodies against poliovirus types 1 2 and 3 These were measured with the neutralization test in tissue culture tubes In the test a constant amount of 30–100 $TCID_{50}$ units of poliovirus in 0 1 ml and the equal volume of serum dilutions in two fold steps starting from 1 4 were used The neutralization occurred at room temperature for six hours and then overnight at +4°C Serum virus mixture was inoculated into tubes of U-cells (a cell line of human amnion cells) Cytopathic effect was read in microscope [illegible] cytopathic effect of [illegible] the titer of corresponding antibodies The results concerning adverse reactions and DTP antibody responses after DTP, and DTP inactivated poliovirus vaccinations will be published separately (14)

RESULTS

Questionnaires concerning the reactions of the poliovirus vaccinations were returned by 119 from group A (63%) and 106 parents from group B (56%) Fever reaction (≥37 5°C measured rectally) was recorded in 14% after the first vaccination and in 19% after the second vaccination Restlessness was reported in 15% and 14% of the children No other marked reactions exceeding the control values were observed, i e , the same kind of symptoms at the time of vaccination in unvaccinated siblings of playmates of the vaccinees The fever reactions started in most children on the day of vaccination and lasted 1–2 days (Fig 1) Fever reactions exceeding 38 5°C were recorded in 5% of the vaccinees Antipyretic medication was given to 4% and 5% of the children and 1% of the parents contacted nurse or physician due to adverse reac-

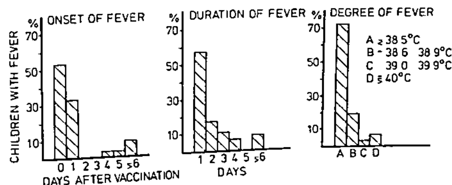


Fig 1 The onset duration and degree of fever reaction after polio virus vaccination

In earlier studies the combination with DTP has had a slight enhancing effect (2)

The major disadvantage of inactivated poliovaccine is that 80–90% of the population should receive complete vaccination program to eradicate the disease. This is the main reason that this vaccine has not been recommended in England or in the United States where only 60–70% of persons are vaccinated (5 13) In Finland, 98% of the children receive complete immunizing program (16) and the wild virus has been eradicated (11) Live oral poliovaccine induces intestinal immunity and spread of immunogenic agent from vaccinated to nonvaccinated persons which are suggested to be its major advantages (12, 13)

In this work we found that inactivated poliovaccine gives good protection against the disease while inducing only a few adverse reactions Our observations and experience in Finland support the concept that inactivated poliovaccine is the vaccine of choice in countries where high vaccination rates can be achieved

ACKNOWLEDGEMENT

This work was supported by a grant from the National Board of Health Finland

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Submitted Sept 7, 1979
Accepted Nov 12, 1979

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Table 3. *The geometric mean titers and percentages of seronegative children with different immunizing schedules*

[illegible] oup B The children received [illegible] All children got IV DTP III [illegible]

Group	Poliovirus type	After two poliovirus vaccinations		After three poliovirus vaccinations	
		Antibody titer	Seronegative (%)	Antibody titer	Seronegative (%)
A	1	1 8	33	1 50	25
B		1 9	36	1 67	21
A	2	1 25	0	1 422	0
B		1 21	14	1 284	4
A	3	1 7	39	1 53	10
B		1 8	32	1 39	11

vaccination (Fig. 1) were due to the possible concomitant infection and not to the vaccination

In another study we have monitored the adverse reactions after different DTP and DTP-inactivated poliovirus vaccinations in these same children using the same questionnaire method (14) We found that DTP and DTP-inactivated poliovaccines induced fever reactions in half of the vaccinees, in 13–28% the fever reactions was ≥38 5°C These findings are in agreement with a previous study by Barkin & Pichichero (1) The onset and duration of fever reactions after DTP vaccination are similar to those of poliovirus vaccination. Inactivated poliovaccine induces clearly less adverse effects than DTP vaccine and no severe reactions are reported Furthermore, the combination of inactivated poliovaccine to DTP vaccine does not markedly increase the frequency and nature of adverse effects The use of this combination reduces the cost of vaccination program

Inactivated poliovaccine has proved to be very safe Live oral poliovaccine is also considered safe but on rare occasions it can cause paralytic poliomyelitis An international investigation in 8 countries revealed 205 cases of paralytic poliomyelitis after 191 million doses of live poliovaccine in 5 years (18) In another investigation the risk of vaccine-associated paralytic disease was estimated to be one case for every 11 5 million persons vaccinated with live poliovaccine in recipients, one case for every 3 9 million in household contacts, and one case for every 22 9 million in community contacts (13) These findings have raised the need to vaccinate unvaccinated parents at the same time as a baby receives the first dose of live oral vaccine (8)

After three doses of inactivated poliovaccine no measurable antibodies were found against poliovirus type 1 in 23%, against type 2 in 2%, and against type 3 in 10% When considering this observation three facts must be stressed 1) Neutralization test for measuring poliovirus antibodies is insensitive and it is possible that the seronegative children would have antibodies if studied with a more sensitive method 2) All the seronegative children in this study were revaccinated and a seroconversion occurred in all children in two weeks after the booster dose indicating their immunity (10) 3) Vaccination program against polio in our country is efficacious since the last patient with paralytic poliomyelitis was seen in Finland in 1964 Furthermore, the new cases of paralytic disease in Sweden 1977 and in the Netherlands 1978 where inactivated vaccine is also used, occurred in persons who had not received vaccinations against poliomyelitis (3, 17) The combination of inactivated poliovaccine to DTP vaccine induced no significant changes in poliovirus antibodies in this study

PARA INFLUENZA PNEUMONIA IN DiGEORGE SYNDROME TWO YEARS AFTER THYMIC EPITHELIAL TRANSPLANTATION

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ABSTRACT Beard, Lorraine J , Robertson, Evelyn F and Thong, Y H (Department of Paediatrics, University of Adelaide and Department of Chemical Pathology, Adelaide Children s Hospital Inc) Para influenza pneumonia in DiGeorge syndrome two years after thymic epithelial transplantation Acta Paediatr Scand 69 403, 1980.—A patient with DiGeorge syndrome developed pneumonia caused by para influenza virus type 3 two years after immunological reconstitution with foetal thymic epithelium There was a transient reduction of mitogen-induced lymphocyte transformation at the time of the pneumonia Although she recovered from the pneumonia, bronchitis persisted and the virus could still be isolated from her pharyngeal secretions 3½ months later

KEY WORDS Para influenza virus type 3, pneumonia, DiGeorge syndrome, thymus transplantation

Very little is known about the subsequent immunological performance of immunodeficient children after reconstitution of immune function One patient with the DiGeorge syndrome (DGS) had normal lymphocyte response to mitogens but low T cell numbers 9 years after transplantation (4) and Good (8) observed that immune reconstitution may be sustained for only a few years but did not present clinical details of the cases Unreconstituted patients with DGS usually die before 2 years of age (9), while even patients with partial DGS syndromes are apt to die suddenly (14) Viral infections present the greatest threat because DGS patients lack cell mediated immunity (9)

A DGS patient we treated with thymic epithelial transplant 2 years ago (20) developed para influenza type 3 penumonia Recovery was slow and the virus persisted for 3½ months The serial immunological studies during and after the infection are reported below

CASE HISTORY

Details of this patient have been previously described (20) Briefly this girl now 3½ years old first presented at age 13 months with pneumonia and hypocalcaemic seizures She had hypoparathyroidism reduced T cell numbers and decreased mitogen induced lymphocyte transformation Other features of the DiGeorge syndrome included hypertelorism short philtrum of the lip right sided aortic arch and aberrant origin of the left subclavian artery Transplantation with foetal thymic epithelium at 15 months of age corrected the lymphocyte response to mitogens but T cell numbers remained subnormal Serum calcium levels have been satisfactorily maintained with dihydrotachysterol and calcium supplements

Approximately 2 years after thymic transplantation she presented with a history and signs suggest ve of pne [illegible]

[illegible] blood examination revealed a hemoglobin of 12 0 g/dl and a peripheral white cell count of 10 7× [illegible]

[illegible]

[illegible] changes appeared before the chest radiograph showed final resolution She was treated with physio-

able 2 *Serial immune function in a patient with DiGeorge syndrome reconstituted with thymic epithelium during para influenzal pneumonia*

	Immune function tests			
	7/7/79	31/1/79[b]	2/4/79	Normal range
erum immunoglobulins				
G (g/l)	8 59	8 69	8 24	5 28–15 12
A (g/l)	0 82	0 52	0 57	0 32–2 00
M (g/l)	0 94	3 1	2 02	0 42–1 74
E (I U /ml)	N D	5 0	17 0	0–16 9
Serum complement				
C 3 (g/l)	0 72	1 18	0 75	0 65–1 50
C 4 (g/l)	0 24	0 59	0 19	0 20–0 40
CH 50 (units/ml)	N D	194	127	95–165
Lymphocyte subpopulations				
T cells (%)	33	41[c]	40	52–70
B cells (%)	16	11	18	8–14
Lymphocyte transformation[a]				
Nil	1 694±393	1 014±158	674±99	
PHA	30 285±8 327	3 742±718	12 483±713	>10 000
PWM	33 295±1 687	1 168±206	6 804±291	>5 000
ConA	36 654±5 282	2 986±504	11 777±1 498	>10 000
Netrophil function				
Iodination (pmol/10^7cells/hr)		4 2	8 7	3 2–7 9
Chemotaxis (mm/3 hr)		1 33	0 83	1 4–2 4
Bactericidal (% *S aureus* killed /2 hr)		94 8	N D	80 5–98 6
Fungicidal (% *T glabrata* killed/2 hr)		91 4	83 7	82 0–96 6

N D not done

[a] Expressed as counts per minute (cpm ± S D) ^{3}H thymidine uptake of triplicate cultures

[b] *Tested during para influenzal pneumonia*

[c] Absolute count 856/mm^3 (normal >1 000/mm^3)

mumps (12), measles (7), and other viruses (13) but not for para influenza Immunological studies in children suffering from para influenzal infection are needed to support this conclusion

The rise in IgM, CH50 and C4 levels can also be regarded as a response to the infection The rise in IgE levels from 5 to 17 I U /ml after the infection is well within the normal limits for her age and not excessive All aspects of neutrophil function were normal, except for the slightly reduced chemotaxis values DGS patients are not known to have associated chemotactic defects The reduced values detected here may be a result of the para influenzal infection, both reduced and elevated chemotactic values have been noted during the course of acute infectious episodes (11

Thymus transplantation has been used in the *treatment of DGS* for more than 10 years, yet only one other report (4) has dealt with the immunological performance of these patients in the longer term Such studies on immunodeficient patients are important, not only because they may help to improve and modify current methods of immunological re constitution, but also may provide an insight into the nature of the immunodeficiency itself

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Table 1 *Serum antibody titres to viruses and mycoplasma in a patient with DiGeorge syndrome reconstituted with thymic epithelium*

Microorganism	Antibody titre 31/1/79	13/2/79
Influenza A	<1 5	<1 5
Influenza B	<1 5	<1 5
Ornithosis group	<1 5	<1 5
Q fever phase 2	<1 5	<1 5
Adenovirus	1 20	<1 5
Respiratory syncytial virus	<1 5	<1 5
Para influenza		
1	<1 5	<1 5
2	<1 5	<1 5
3	<1 5	1 40[a]
Mycoplasma pneumoniae	1 40	1 40

[a] Significant rise in titre

therapy and Amoxycillin and was discharged after 6 days in hospital Serology showed a significant rise in titre to only para influenza virus type 3 from <1 5 to 1 40 (Table 1) For several months after this episode of pneumonia this girl has had an annoying nocturnal cough and wheeze which has been helped considerably with oral salbutamol

MATERIALS AND METHODS

The serum immunoglobulins (A G M) and complement (C3 C4) concentrations were measured by radial immunodiffusion using commercial plates (Behringwerke W Germany) serum IgE by the Phadebas kit (Pharmacia Sweden) total haemolytic complement by the lysis of sheep red blood cells (12) Lymphocytes and neutrophils were purified from heparinized blood by a one step gradient sedimentation procedure (6) T cells counted by spontaneous rosetting with sheep red blood cells (15) and B cells by immunofluorescence of surface antigens using anti human antibody (1) Lymphocyte transformation to mitogens was assessed by a micro culture method (21) Quantitative neutrophil iodination was determined by a microassay technique (18) neutrophil chemotaxis by migration under agarose (19) bactericidal activity by reduction of *Staphylococcus aureus* colony counts (17) and fungicidal capacity by reduction of *Torulopsis glabrata* colony counts (16) Viral antibody titres on acute and convalescent sera were determined by complement fixation

RESULTS

The significant rise in antibody titre to para influenza virus type 3 only (Table 1) and the isolation of this virus from a pharyngeal swab, taken together with the clinical and radiological findings indicate that this patient had contracted peneumonia caused by para influenza virus type 3

The results of sequential immune function testing are shown in Table 2 There was an increase in IgM at the time of the pneumonia while other immunoglobulin levels remained within the normal range Likewise, serum C4 and CH 50 increased at this time T cell numbers which had, until this illness, remained in the 20–30% range rose to around 40% and have remained at this level

However, the most interesting finding was the marked reduction in mitogen induced lymphocyte transformation at the time of the pneumonia, with a subsequent return to normal values The response to phytohaemagglutinin (PHA) fell to only 3 742±718 (cpm±S D) with a stimulation index of only 3 7 Two months later it had risen to 12 483±713 (cpm± S D) with a stimulation index of 19 4 Responses to other mitogens paralleled those to PHA

DISCUSSION

Para influenza viruses are imprtant causes of respiratory infections in children (3) The most virulent serotype appears to be type 3 which has caused fatal pneumonia in children with immunodeficiency (5 10) Thus the ability of this DGS patient to recover from para influenza type 3 pneumonia would indicate that adequate immunological responses were present However the delay in recovery and persistence of virus for 3½ months would suggest that her immunocompetence may not be completely normal This conclusion is borne out by the persistently low T cell numbers (absolute count <1 000/mm³) in the past, and only 856/mm³ during this acute illness

Lymphocyte mitogen responses however, were raised to normal levels after thymic epithelial transplantation (20) The markedly depressed responses during this episode of para influenza pneumonia may be best explained on the basis of immunosuppression by the viral infection (13), this has been documented for

Acta Pædiatr Scand 69 407–409, 1980

ANTIBODIES TO CYTOMEGALOVIRUS AMONG PERSONNEL AT A CHILDREN'S HOSPITAL

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ABSTRACT Haneberg, B , Bertnes, E. and Haukenes, G (Department of Paediatrics and Department of Microbiology, University of Bergen, Norway) Antibodies to cytomegalovirus among personnel at a children s hospital Acta Paediatr Scand, 69 407, 1980.—Among 161 individual personnel at Children's Hospital, serum complement fixing antibodies were found more frequently (77 %) in those who were in close contact with the patients than (39 %) in those with less contact This difference was most pronounced in the young age group Most (80 %) of the personnel at an age of 30 years or older, were seropositive The results indicate that young personnel, working in close contact with infants and children in hospital, are at great risk of acquiring cytomegalovirus infection Student nurses, being exposed to these patients only for a few weeks, also were at risk of being infected Seronegative pregnant women, working in a children s hospital, should therefore take measures to protect themselves from close contact with the patients since virus excretion occurs frequently, even without clinical signs of CMV infection

KEY WORDS CMV antibodies, children s hospital

Intrauterine cytomegalovirus (CMV) infections may have serious implications for the infant and child (3, 5) Infected infants are also delivered by women who had their primary infection prior to the actual pregnancy (4)

Among the patients at the University Children's Hospital in Bergen, and at other hospitals (1), there are many CMV excretors in all age groups This made us concerned about the risk for primary infection among the female nursing personnel since most of them are at a child bearing age and usually continue their jobs during pregnancy We, therefore, have registered serum antibodies to CMV among the personnel and student nurses working in more or less close contact with the patients, and have tried to analyse their risk of acquiring an infection

MATERIALS AND METHODS

Fifty three nurses 82 nurse s aids 13 ward aids and 13 laboratory technicians at University Children s Hospital were included in the study They were working at 5 different wards newborn intensive care unit regular infant unit units for either pre school or school children and infectious disease unit Persons that had been working at this hospital for less than 6 months were not included In addition 163 and 124 student nurses were tested at the entry of the nursing school and during their service at Children s Hospital respectively

bryonal lung fibroblast cultures infected with the CMV strain AD 169 Tests for IgM antibodies and antibodies for CMV early antigen were carried out by indirect

RESULTS

The personnel working at the units for infants, was largely seropositive (Table 1) On the other hand, less than half of the personnel at the unit for school children was seropositive This may indicate a greater risk of CMV-infection when caring for small infants

On the basis of an assumed risk of infection by being in close contact with the patients, such as during feeding and handling of diapers, and by being in contact with stools, urine and

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Submitted Aug 17 1979
Accepted Oct 29 1979

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these could IgM- antibodies and antibodies to CMV early antigen be demonstrated It is also noteworthy that the 6 seroconverters belonged to the first 35 seronegative students retested Of the later ones retested, none showed seroconversion

DISCUSSION

The results of the present investigation indicate that there is a considerable risk of acquiring CMV infection for those working in close contact with patients at a children's hospital Others have not detected such a risk (3) although differences in hygienic precautions may explain the discrepancy For example, it is common in this hospital to see the personnel kiss the drooling infants while feeding them

The demonstration of seroconversion among student nurses, working only a short time in the Children s Hospital, also gives an indication of the risk of infection there The presence of IgM antibodies and antibodies to CMV early antigen confirms a recent primary infection in one of them On the other hand, the abscence of these antibodies in the other sera does not exclude a primary infection Also, the finding of seroconverters among the first students studied, and not among students tested at a later date, when more attention was given to the risks involved, points to the fact that CMV infection can be prevented if some hygienic precautions are taken

If some of the personnel without detectable CMV antibodies are, or might be, pregnant, they should therefore take precautions to avoid close contact with patients or their blood or excretions It is not practicable only to avoid contact with known virus excretors, as proposed by others (2), since most excretors have no other signs of CMV-infection (1, 3).

ACKNOWLEDGEMENTS

We are grateful to the Employee s health department for obtaining some of the sera and to Ivar Ørstavik, M D Ullevål Hospital Oslo and Peter Csango, M D at the National Institute of Health Oslo, for examining the sera for IgM antibodies and antibodies to CMV early antigen, respectively

This study was supported, in part, by Åndssvakesakens Forskningsfond

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Submitted Aug 13, 1979
Accepted Oct 22 1979

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Table 1 *Number of personnel at the different units at Children's Hospital with serum antibodies (titer ≥4) to CMV*

Unit	Number of personnel	
	Total	CMV positive
Infant intensive care	29	21 (72%)
Infants regular	45	36 (80%)
Pre school children	27	17 (63%)
School children	25	10 (40%)
Infectious diseases	22	12 (54%)
Laboratory	13	9 (69%)

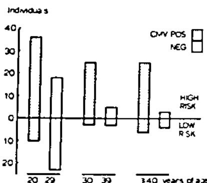


Fig 1 Number of personnel at Children's Hospital with and without detectable serum antibodies to CMV divided into 3 age groups and separated by the horizontal line into a high risk group above and a low risk group below this line

blood, the personnel was divided into two groups, a high risk and a low-risk group In the high risk group were included all nurse's aids, nurses at the regular infant unit and laboratory technicians In the low-risk group were included the nurses at the other units as well as the ward aids, who were not having regular close contact with the patients It is evident that a much higher proportion of the personnel in the high-risk group was positive than among those in the low risk group (Table 2) Thus, close contact with patients or excrements, urine or blood from them, regardless of the patients' age, seemed to carry increased risk of acquiring a CMV-infection

Increasing age of the personnel was accompanied by higher frequency of sero positivity In the youngest age group 53% were seropositive while 80% were seropositive in the age group 30 years or older (Fig 1)

Table 2 *Number of personnel at Children's Hospital belonging to either high risk or low risk groups with serum antibodies (titer ≥4) to CMV*

The high risk group included all nurse's aids nurses at regular infant unit and laboratory technicians The low risk group included the rest of the nurses and the ward aids

	Number of personnel	
	Total	CMV positive
High risk group	112	86 (77%)
Low risk group	49	19 (39%)

Among the youngest, 20 to 29 years of ag[e] there was a marked difference between t[he] high- and low risk groups, 66 and 30% r[e]spectively, had antibodies to CMV

Among the student nurses 40% were ser[o]positive for CMV when they entered the nur[s]ing school at a mean age of 20 6 years (ran[ge] 18–35 years with only 4% of the students bei[ng] older than 25 years) Two years later, befo[re] they started their rotating clinical training [at] the Children's Hospital, 42% were seropos[i]tive Among 64 seronegative students, wh[o] were retested within 2 months after the[y] had completed their 2 months long service showed at least a 16-fold increment in seru[m] antibody titers, indicating a recent CM[V] infection (Table 3) However, only in one c[ase]

Table 3 *Seroconverters for antibodies to CMV among 64 seronegative student nurses working for about 2 months at Children's Hospital*

Unit of service	Number of students	
	Studied[a]	With sero conversion
Infant intensive care	5	0
Infant regular	30	3
Pre school children	16	1
School children	19	1
Infectious diseases	18	1

[a] Some students worked at 2 different units so that the total number studied exceeds the number of students

Acta Pædiatr Scand 69 411–413, 1980

DEFECTIVE BACTERICIDAL FUNCTION OF POLYMORPHONUCLEAR NEUTROPHILS IN CHILDREN WITH MEASLES

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ABSTRACT Yetgin, S , and Altay, C (Department of Paediatrics, Children's Medical Center, Hacettepe University, Ankara, Turkey) Defective bactericid function of polymorphonuclear neutrophils in children with measles Acta Paediatr Scand, 69 411, 1980 Killing ca [illegible] the measles The serum opsonic activity was found to be normal

KEY WORDS Bactericidal capacity, serum opsonic activity, polymorphonuclear neutrophils

Measles is one of the childhood infections which can cause death because of secondary bacterial complications (5) Impairment of cell mediated immunity and a defect in neutrophil motility have been reported in these patients (1, 4) The present study was designed to evaluate the bactericidal capacity of polymorphonuclear neutrophils (PMN), and serum opsonic activity during the course of the disease

MATERIAL AND METHOD

Fifteen children with measles between the ages of 11 months and 5 years were studied The patients were selected from those who did not have anemia malnutrition and were not being given any medication at the time of the examination A diagnosis of measles was based on clinical findings Patients who had possible bacterial infection in addition to measles by clinical cultural X ray examination and hematologic findings were considered separately in some statistical evaluations The first day of the exanthem was recorded as Day 1 of the infection One case was examined one day prior to exanthem and was recorded as Day 0 There were two groups of controls 5 children whose condition suggested bacterial bronchopneumonia based on clinical hematological and X ray findings and 15 healthy age matched children

The bactericidal test of Quie et al (8) was used in preparation of bacteria leukocytes and medium *Staphylococcus aureus* catalase (+) was used (2–3 bac [illegible] The percentage of viable bacteria remaining after 120 min of incubation was the measure of bactericidal capacity Controls were studied at the same time

Serum opsonic activity was essentially estimated by the method of Chandra (2) The total number of bacteria present in the patient s serum was compared with the number obtained from the patient s PMN cultured in a medium containing normal serum after 20 min of incubation

Hematological values were obtained by standard methods (10) Chest X-rays were taken of all patients

Four patients were studied again on the 10th day of the infection

RESULTS

The hematological and clinical data of the patients are summarized in Table 1 Seven of the 15 patients had bronchopneumonia in addition to measles One of the 7 patients had otitis media which grew *Staph aureus* on culture, 4 others had positive bacterial throat cultures Viral studies were not done, but these patients were thought to have bacterial bronchopneumonia because of raised neutrophil counts, with toxic granulation, all recovered with antibiotic treatment

The bactericidal capacity of PMN in patients with measles was lower than that of controls In patients with measles and bronchopneumonia, it was lower than with measles alone, but the difference was not statistically

bactericidal defect than the measles patients alone

In 4 cases abnormal killing function of PMN returned to the normal level on the 10th day of infection These findings suggest that measles virus may cause abnormality in PMN function at the early stages of the disease Viruses cause some metabolic and morphologic changes in cells The reduction of PMN glycolysis by influenza virus has been shown (3)

In spite of the fact that antibody response to some bacteria has been found decreased in patients with measles humoral immunity was within normal limits (9) Serum opsonic activity against *S aureus* was detected to be within normal limits in our study

Defective bactericidal function of PMN found in this study may explain why measles render patient prone to bacterial infection However it is not known how the virus affects bactericidal functions of the PMN

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Submitted July 4 1979
Accepted Oct 30 1979

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Table 1 *Some of the laboratory and clinical data*

Case no	Hb (g/l)	PMN count (10⁹/l)	Chest X Ray	Examination day after	Bactericidal capacity of PMN (% remaining viable bacteria) Patient	Control
Measles						
1	126	3 30	N[a]	1	28	14
2	116	3 63	N	1	25	18
3	110	3 14	N	1	25	14
4	115	2 25	N	2	21	18
5	127	0 70	–	3	19	9
6	115	2 20	N	1	30	9
7	112	3 90	N	1	35	10
8	127	3 30	N	1	55	20
Measles with BP[b]						
9	119	4 96	BP	2	25	19
10	110	4 50	BP	0	54	8
11	129	4 65	BP	1	53	8
12	112	3 11	BP	2	30	15
13	120	4 10	BP	1	55	19
14	115	4 99	BP	1	15	12
15	116	7 53	BP	3	28	19
Mean	117	3 57			33 3	14 2
± 1 S D	7	4 14		13 8	4 5	

[a] Normal
[b] Bronchopneumonia

significant The mean of bactericidal capacity of PMN in controls with bronchopneumonia was also found to be decreased (Table 2)

Serum opsonic activity was normal in all groups studied the bactericidal capacity returned to the normal level in 4 cases on the 10th day of the infection

DISCUSSION

Abnormality in cell-mediated immunity has long been known in measles (4) It was recently reported that motility of PMN was defective in children with measles (1) A defect in motility and abnormal phagocytic function has als been reported in PMN obtained from norm adult individuals incubated with influenza v rus (6) All of these studies indicate that im mune system is influenced in patients wit measles

In our study of cases of measles the killin capacity of PMN was shown to be defective Defective bactericidal function of PMN ha been reported in cases with bacterial infectio (7) A similar result was obtained in our stud from controls with bronchopneumonia Thi might explain why the patients who had bot measles and bronchopneumonia had a greate

Table 2 *Mean values of PMN function*

	Bactericidal capacity (%remaining viable bacteria)			Serum opsonic activity (Bacteria in 10⁶ PMN × 10⁻⁵)		
Measles	(8)[a]	30 0±11 1	$p<0$ 001			
Measles + bacterial infection	(7)	37 1±16 4	$p<0$ 001			
Total number of patients	(15)	33 3±13 8	$p<0$ 001	(11)	5 3±0 9	$p>0$ 0
Controls with bronchopneumonia	(5)	224 2±7 0	$p<0$ 01			
Healthy controls	(15)	14 2±4 5		(11)	4 8±1 5	

[a] Figures in parentheses refer to the number of cases examined

Acta Pædiatr Scand 69: 415–418, 1980

CASE REPORT

MENINGITIS IN A NEWBORN INFANT CAUSED BY *MYCOPLASMA HOMINIS*

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ABSTRACT. Hjelm, E ,
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Sweden, and the Institute of Medical Microbiology, University of Aarhus, Aarhus, Denmark).
Meningitis in a newborn infant caused by *Mycoplasma hominis* Acta Paediatr Scand, 69, 415, 1980.—When 10 days old an infant born after 34–35 weeks of gestation developed meningitis with pleocytosis and a low glucose concentration in the cerebrospinal fluid. *Mycoplasma hominis* was recovered from the cerebrospinal fluid and treatment with doxycycline was given The strain was later found to be resistant to tetracycline. After institution of lincomycin, cultures for mycoplasmas were negative The infant, who during the course of the meningitis had developed a transient increase in intracranial pressure, was healthy and normally developed at the age of one year.—This is the first report on an infection caused by a tetracycline-resistant strain of Mycoplasma hominis.

KEY WORDS Meningitis, mycoplasma hominis, newborn infants, tetracycline resistance

Mycoplasma hominis (*M hominis*) can be recovered from the urethra and/or cervix of more than every second young adult woman with signs of genital infection, but significantly less often from healthy females (15, 16) The organism may give rise to ascending genital infections, resulting in acute salpingitis (14) Occasionally, *M hominis* may also be isolated from the respiratory tract (18, 19)

The newborn infant may be colonized by *M hominis* This occurs significantly more often in infants of low birth weight (11) Systemic infections with *M hominis* of intrauterine origin in stillborn infants have been reported (9) There have also been a few cases of subcutaneous abscesses and of conjunctivitis associated with *M hominis* infection in newborns (7, 10, 22)

Pleuropneumonia-like organisms (PPLO) or organisms identified as *M hominis* have been isolated in a few cases from both brain abscesses and cerebrospinal fluid (CSF) in adults (20), children (3) and newborns (2, 6, 25, 26).

In the present study, meningitis caused by a tetracycline-resistant strain of *M. hominis* in a newborn infant is described.

CASE REPORT

A male infant was born after 34–35 weeks of gestation, 48 h after spontaneous rupture of the membranes, with a birth weight of 2 2 kg The Apgar score at 1 min was 8 The placenta and the amniotic fluid appeared normal

On the first day of life the infant showed signs of a systemic infection, and treatment with ampicillin was started However, bacterial cultures from the blood, urine and CSF and from gastric aspirate proved negative No pathogenic bacteria were demonstrated in specimens from the nasopharynx The lungs appeared normal at radiography

During the second day an exchange transfusion was performed because of haemolytic disease (B immunization)

The infant progressed well until the 10th day, when he became irritable and his rectal temperature was 38°C Examination of CSF showed pleocytosis (Table 1) As an infection with an ampicillin resistant organism was sus-

Table 2 *Antibiotic treatment given to the infant*

Age (days)	Drug	Dosage (mg/kg/day)	Doses/day and route of admin
1–10	Ampicillin	100	2 i v
11 20	Gentamicin	3–4	2 i m
	Carbenicillin	100	2 i v
25–34	Doxycycline	4	1 i v
35–49	Lincomycin	30	3 i m
50–	*No antibiotic treatment*		

›bit globulin (Dakopatt Copenhagen Denmark) was ed

Tests for antibody to M hominis Tests for IHA anti ›dies to *M hominis* were carried out by the method of rogsgaard Jensen (12)

Antibiotic susceptibility tests The method used for de rmining the MIC of antibiotics for the isolated strains *M hominis* has been described elsewhere (13)

Antibiotic concentrations in plasma and CSF were ›easured by the micromethod described by Jalling et (8)

DISCUSSION

n the reported case irritability an increased number of white blood cells and a low glucose concentration in the CSF indicated an infection of the central nervous system (CNS) Routine cultures and serological tests did not reveal the organism causing the infection These tests were then extended, and mycoplasmas were isolated from the CSF Some strains of mycoplasmas may be isolated on conventional blood agar plates, while others, as in the present case, cannot be recovered unless media especially designed for the culture of mycoplasmas are employed

Appropriate antibiotic treatment was delayed by the fact that the isolated strains of *M hominis* were resistant to doxycycline, known hitherto as the drug of choice in *M hominis* infections When the treatment was changed to lincomycin, CSF cultures for mycoplasmas were negative after only one day

Attempts to identify the isolated strains of *Mycoplasma* by growth inhibition tests failed when a commercially available antiserum to *M hominis* was used, as no growth inhibition zones were obtained However, in immunofluorescence tests the isolated strains were identified as *M hominis* The same identification was made when another high titer antiserum to *M hominis* was used in growth inhibition tests, although smaller growth inhibition zones were obtained than are generally found when testing tetracycline susceptible strains of this species

Further studies (5) of the tetracycline-resistant strains revealed that they probably contained plasmids and that the chemical composition of their cytoplasmic membrane differs markedly from that of tetracycline susceptible strains of the same species Both qualitative and quantitative differences with regard to the cytoplasmic membrane lipids and proteins were observed This might explain the decreased susceptibility to tetracyclines, as it is known that bacterial resistance to such drugs may be associated with alterations of the chemical composition of these structures, resulting in a reduced passage of tetracyclines into the cell The small growth inhibition zones obtained with the high titer antisera and the failure of the commercial antisera to *M hominis* used to produce any such zone might also be explained by such alterations, the difference in the effect of these two antisera is probably due to their different titers of homologous antibodies to *M hominis*

Tetracycline resistance in clinical isolates of *M hominis* has not been reported previously For the strains isolated from the infant the MIC of tetracycline was 32 μg/ml When testing 100 strains of *M hominis* recovered from the female genital tract, we found that they had tetracycline MICs of 0 1–0 8 μg/ml (27)

Although humoral immune mechanisms are not fully developed in newborns (17), a high titer of serum IHA antibodies to *M hominis* was found in our patient as early as on the 24th day of life The highest titer observed, 1/1280, is comparable to the highest titers of such antibodies demonstrated in human beings, that is, in females with acute salpingitis (14) The fact that no IHA antibodies to

27 80_873

Table 1 *Some laboratory findings in the newborn infant with meningitis caused by Mycoplasma hominis*

	Examination at age (days)									
	10	19	24	28	34	36	40	45	58	72
CSF										
White blood cells (10^6/l)										
Polymorphonucl	220	32	314	1 090	214	84	2	2	1	
Mononucl	800	134	304	730	414	271	204	76	10	
Erythrocytes (10^6/l)	650	195	8	3	10	3 400	20	36	0	
Protein (g/l)	1 75	1 70	1 74	2 24	2 47	3 30	1 92	1 30	0 77	
Glucose (mmol/l)	1 3	0 9	0 5	0	0	0 8	1 5	2 3	1 5	
Culture for *M hominis*	ND[a]	Pos	Pos	Pos	Pos	Neg	Neg	Neg	Neg	
Blood glucose (mmol/l)	3 6	5 1	4 3	5 6	N D[a]	5 4	N D[a]	4 5	N D[a]	
Serum titer of IHA antibodies to *M hominis*		1/40	1/1 280	1/1 280			1/640	1/640		1/3 0

[a] N D = not done

pected the treatment was changed to carbenicillin and gentamicin The general condition then improved and the CSF white cell count decreased but the CSF glucose concentration was still very low Repeated bacterial cultures from CSF were all negative

When the infant was 19 days old CSF was cultured for mycoplasmas As this culture was positive the treatment was changed to doxycycline (Table 2) but this had no effect on the pleocytosis or the glucose concentration in the CSF Mycoplasmas were thereafter isolated from CSF on several occasions The minimum inhibitory concentration (MIC) of different antibiotics for the strains were 32 µg/ml for ampicillin carbenicillin chloramphenicol doxycycline and gentamicin and 0 2 µg/ml for lincomycin

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mycin in the CSF was less than 1 µg/ml while the plasma concentration reached a peak level of about 12 µg/ml The pleocytosis gradually disappeared and the CSF glucose concentration became normal

The organisms isolated from the infant were subsequently identified as *M hominis* It was also demonstrated later that the titer of indirect haemagglutination antibodies (IHA) to this organism had changed significantly during the course of the disease

The infant s general condition was fairly good throughout the illness Fever was only present initially He was able to suck and gained weight but was irritable Up to the age of 35 days the head circumference was pathologically increased Computed tomography revealed dilated lateral ventricles After institution of lincomycin the head circumference returned to normal Repeated echo encephalograms showed that the lateral ventricles became normal in size At one year of age a neurological examination disclosed no abnormalities and the infants seemed to have developed normally in all respects

There were no findings to indicate infection with any other organism than *M hominis* CSF cultures for bacteria including mycobacteria were negative and no v

and *Toxoplasma gondii* WR VDRL Kleine TPI and FTA ABS tests were also negative

The mother who was of Jordanian origin had arrived from the Middle East one week before delivery She had not attended any ante natal clinic There was no history of illness during pregnancy A strain of *M hominis* was isolated from the mother s urethra This strain showed the same antibiotic susceptibility pattern as the strains isolated from the CSF of the infant No IHA antibodies to *M hominis* were found in the mother s serum

Laboratory investigations

Bacterial cultures CSF was cultured on blood and haematin agar plates which were incubated in a 10% CO_2 atmosphere Fresh blood agar plates were also inoculated and incubated at 37°C for 2 days in anaerobic jars (Gas Pak BBL Maryland USA) In addition CSF was inoculated into aerobic and anaerobic blood culture bottles (SBL Stockholm Sweden) The bottles were inspected daily for 6 days

Cultures for mycoplasmas and ureaplasmas The specimens were cultured on solid and liquid *Mycoplasma* media (4) and on two *Ureaplasma* media namely on Ureaplasma differential agar (A7) (24) and in a urease broth (U9) (23) The agar plates were incubated at 37°C in an atmosphere of 90% N_2 and 10% CO_2 After 6 days of incubation at 37°C the liquid media were subcultured on solid medium of the same composition

The isolated strains of *Mycoplasma* were identified by indirect immunofluorescence tests of isolated colonies on agar plates (21) and growth inhibition tests (1) In the immunofluorescence tests fluorescein conjugated anti

Acta Pædiatr Scand 69, 419–424, 1980

CASE REPORT

VASOACTIVE INTESTINAL POLYPEPTIDE (VIP)-PRODUCING GANGLIONEUROMA IN A CHILD WITH CHRONIC DIARRHEA

LARS PETER HANSEN, HANS T LUND, JAN FAHRENKRUG and HELMER SØGAARD

From the Department of Paediatrics, Central Hospital, Randers, the Department of Clinical Chemistry Bispebjerg Hospital, Copenhagen, and the Department of Pathology, Municipal Hospital, Århus, Denmark

ABSTRACT. Hansen, L. P , Lund, H. T., Fahrenkrug, J. and Søgaard, H. (The Department of Paediatrics, Central Hospital, Randers, The Department of Clinical Chemistry, Bispebjerg Hospital, Copenhagen, and the Department of Pathology, Municipal Hospital, Århus, Denmark) Vasoactive intestinal polypeptide (VIP)-producing ganglioneuroma in a child with chronic diarrhea. Acta Paediatr Scand, 69 419, 1980 In a 2½-year-old girl with persistent watery diarrhea a retroperitoneal tumour and lymph node composed of benign ganglioneuroma cells were found The histological picture was compatible with an original metastasizing neuroblastoma which has passed through a stage of late maturation Electron microscopy of the ganglioneuroma showed cytological evidence of increased secretory activity. The tumour contained large amounts of vasoactive intestinal polypeptide (VIP) and preoperative plasma concentration of this peptide was increased Postoperatively the VIP concentration was normalized, and the diarrhea ceased. Analysis of catecholamine metabolites in urine was normal. The findings suggest that the watery diarrhea was due to the benign VIP producing neural crest tumour.

KEY WORDS. Chronic diarrhea, ganglioneuroma, vasoactive intestinal peptide

The association between chronic diarrhea and neural crest tumour was first described in 1952 (12) The pathogenesis of the diarrhea is yet unknown but the cessation of diarrhea after removal of the neoplasm suggests that humoral substances such as catecholamines produced by the tumour may be responsible (14, 25, 32) Excretion of catecholamines is in some cases normal (8, 9, 13, 23) Consequently, other agents have been proposed Among these vasoactive intestinal polypeptide (VIP) has gained the strongest support (7, 13, 18, 24, 29)

VIP, a 28-aminoacid peptide, isolated from porcine intestine (28), has a broad spectrum of biological actions (19, 26), among these stimulatory effect on intestinal secretion (1) ...ted plasma and tumour levels of VIP ... been demonstrated in patients with WDHA (watery diarrhea hypokalaemia-achlorhydria) syndrome associated with pancreatic or neurogenic tumours (2, 6, 7, 13, 18, 22, 24, 27, 29, 30) Here we report a case which gives additional confirmation of the involvement of VIP in diarrhea associated with neurogenic tumours

CASE HISTORY

A 2½ year-old girl was admitted with a 17 month history of persistent diarrhea Family history was negative Pregnancy, birth and psychomotoric development were normal Apart from diarrhea she had *previously been healthy* The mother had noticed a faint rash for several months which remained unchanged until admission The stools were described as watery with no blood or mucus, occurring 4–6 times daily Dietary precautions and constipating agents had no effect on the diarrhea Weight gain was normal The general condition of the child was good with

M. hominis were found in the mother's sera, in spite of isolation of the organism from the lower genital tract, is in conformity with the results of earlier studies (14)

From an epidemiological standpoint the demonstration of tetracycline-resistant stains of *M. hominis* may be of the same significance as the recent demonstration of β-lactamase-producing strains of gonococci.

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Submitted July 2, 1979
Accepted Nov 6 1979

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Acta Pædiatr Scand 69 419–424, 1980

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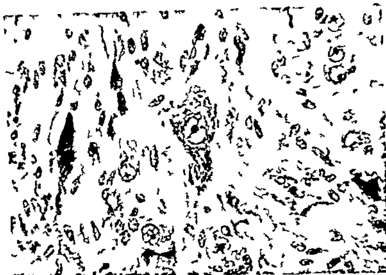


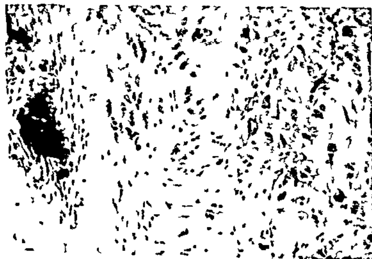
Fig 2 Ganglioneuroma Well different ated gangl on cells Schwann cells and nerve fbres in a vacuolated stroma Hematoxylin eosin ×400

e 10 pmol/l the day after operat on 9 5 pmol/l three months later and 3 5 pmol/l at a control 9 months later The extirpated gangl oneuroma was found to contain mas s ve amounts of VIP 30 000 pmol/g wet we ght (101 μg/g)

DISCUSSION

There is no symptomatology specific for the neurogenic group of retroperitoneal tumours Chronic diarrhea resulting in hypokalaemia (7 13 18 23) as a feature in neurogenic tumours has been reported in less than 10% of the cases (25 32) The occurrence of cutaneous symptoms has also been recorded in some of the previous cases (8 10 12, 25)

The findings reported here suggest that VIP was the humeral mediator of the patient s symptoms (diarrhea hypokalaemia, and rash) based on the following lines of evidence 1) *The circulating level of VIP was markedly* raised preoperatively 2) The tumour extract contained large amounts of VIP 3) Electron microscopy demonstrated increased secretory activity in the tumour 4) Extirpation of the tumour resulted in a normalization of the



F g 3 Regional lymph node containing mature gangl oneuroma *Left* A calcium deposit probably degenerated *neuroblastoma* t ssue from metastasis Hematoxylin eos n ×150



Fig 1 Selective arteriogram of the third right lumbar artery The well-demarcated and *richly vascularized* tumour mass containing moderately ectatic and irregular vasculature supplied from the third *right lumbar artery* Via anastomosis retrograde filling of the two neighbouring arteries is seen

were normal for age Temperature was 37 3°C Pulse rate 104 beats per minute and blood pressure 110/60 mmHg

An indistinct fine spotted rash was seen on the face neck upper arms and upper part of the chest The abdomen was slightly distended with dilatation of the cutaneous veins Deep in the upper right quadrant a small firm partly movable indolent mass was palpated medial to the right kidney Rectal examination revealed a rectum empty of solid stool and provoked a squirt of watery faeces Further physical examination revealed no abnormalities

Laboratory investigations and roentgenographic findings

Haemoglobin ESR white cell count differential count serum protein electrophoresis urine vanilmandelic acid and homovanillic acid kidney and liver function tests were all normal Serum electrolytes were normal except for a slight hypokalaemia (3 0 mmol/l) No tumour cells were present in bone marrow aspirate Urine and stool cultures were negative X ray of the kidneys showed irregular calcifications to the right of the third lumbar transverse process A slight malrotation of the right sided pyelogram and a minor displacement of the lower kidney pole suggesting a retroperitoneal tumour close to the lower pole of the right kidney were noticed Aortography with selective arteriograms of celiac right renal and right third lumbar arteries showed a well-demarcated and richly vascularized tumour supplied from the third lumbar artery (Fig 1)

Pathological findings

At laparotomy a retroperitoneal firm encapsulated tumour weighing 78 g and measuring 8×5×5 cm was extirpated *in toto* together with a lymph node from the area around the right renal vein which compressed the inferior caval vein

Histological examination of the tumour and regional lymph node showed in all sections a completely matured benign ganglioneuroma (Fig 2) which suggested that original neuroblastoma both in the main localization and in the metastasis had undergone total maturation (Fig 3)

Electron microscopy

Scattered mature nerve cells with peripherally situated granular endoplasmatic reticulum were found The Schwann cell was the most frequent cell type (Fig 4A) Its cytoplasm contained a large number of small invaginated axons The intercellular space contained a multitude of cytoplasmic structures of which some were extensions from Schwann cells others axons with typical neurofibrils Many Schwann cells had engulfed other cells containing large numbers of vesicles of two main types (Fig 4B) One was small round about 800 Å in diameter with a highly electron-dense core occasionally surrounded by a rather narrow clear halo These were interpreted as secretory granules Such granules were also sometimes present in axons The other type was larger and contained heterogeneous membranous material often with a laminated myelin like appearance These bodies were thought to represent phagolysosomes containing remnants of degenerated cytoplasmic structures and possibly lipid Conceivably these cells had engulfed material from necrotic cells or secretion products deliberated by the maturation process of the tumour

Follow up

The patient s postoperative course was uneventful The diarrhea ceased bowel movement remained normal and the rash disappeared One year later the patient was symptomless

VIP determinations

The concentrations of VIP in plasma and in an acid ethanol extract of tumour tissue were measured radioim

tive controls all showed values within the normal range

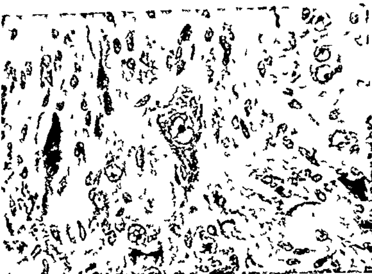


Fig 2 Ganglioneuroma Well differentiated ganglion cells Schwann cells and nerve fibres in a vacuolated stroma Hematoxylin eosin ×400

e 10 pmol/l the day after operation 9 5 pmol/l three months later and 3 5 pmol/l at a control 9 months later The extirpated ganglioneuroma was found to contain massive amounts of VIP 30 000 pmol/g wet weight (101 μg/g)

DISCUSSION

There is no symptomatology specific for the neurogenic group of retroperitoneal tumours Chronic diarrhea resulting in hypokalaemia (7 13 18 23) as a feature in neurogenic tumours has been reported in less than 10% of the cases (25, 32) The occurrence of cutaneous symptoms has also been recorded in some of the previous cases (8, 10, 12, 25)

The findings reported here suggest that VIP was the humeral mediator of the patient's symptoms (diarrhea, hypokalaemia, and rash) based on the following lines of evidence 1) The circulating level of VIP was markedly raised preoperatively 2) The tumour extract contained large amounts of VIP 3) Electron microscopy demonstrated increased secretory activity in the tumour 4) Extirpation of the tumour resulted in a normalization of the

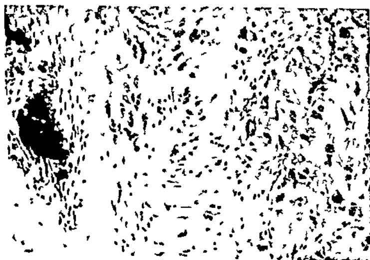


Fig 3 Regional lymph node con tain ng mature ganglioneuroma *Left* A calcium deposit probably degenerated neuroblastoma tissue from metastasis Hematoxylin eosin ×150



Fig 4 (A) Electron micrograph of the tumour with two Schwann cells (their nuclei are marked by *S*) Cytoplasm from other Schwann cells containing invaginated axons (*arrows*) To the left the cytoplasm of a Schwann cell which contains a great number of small and large vesicular structures with electron dense content ×15 000 (B) Electron micrograph of a Schwann cell engulfing another cell which contains two types of vesicular structures One type is small and round (*encircled*) probably representing secretory granules They are occasionally surrounded by a clear halo Such granules are also present in axons (*bottom left*) The other type is larger and contains heterogenous material sometimes with myelin like structures They are probably phagolysosomes *MI* degenerated mitochondrias Arrows indicate the thin layer of Schwann cell cytoplasm surrounding granulated cell ×26 000

plasma VIP concentration and relief of the patient s symptoms, and 5) The biological actions of VIP accord well with the symptoms, and prolonged infusions of VIP in experimental animals provoke the clinical syndrome (watery diarrhea and flushing) at plasma levels comparable to those observed in the present case (21)

The occurrence of VIP in neurogenic tumours is not surprising, considering the occurrence of VIP in neurons which are widely distributed in the body (5, 15, 16, 17) A similar histological picture as in the present case was noticed in two out of more than two hundred cases of neuroblastomas (11) It is well established that maturation of neuroblastic tumours as well as spontaneous regression of neuroblastoma may occur (3, 20, 31) Ganglioneuro blastoma and ganglioneuroma are considered as stages in the process of maturation of neuroblastoma, and therefore careful search for histological evidence for differentiation is compulsory, as maturation of primitive cell types is known to improve prognosis of neuroblastic tumours (20)

Since the symptomatology of the neurogenic tumours is variable, it is possible that the ap plication of VIP measurements may prove to be useful as a diagnostic test as well as monitoring the effect of therapy and to predict regrowth of the tumours

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Submitted April 30 1979
Accepted Oct 29, 1979

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Acta Pædiatr Scand 69, 425–426, 1980

CASE REPORT

EFFECT OF TOLAZOLINE IN PULMONARY HEMORRHAGE IN THE NEWBORN

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ABSTRACT. Markestad, T. and Finne, P. H. (Department of Paediatrics, University of Bergen, Bergen, Norway) Effect of tolazoline in pulmonary hemorrhage in the newborn. Acta Paediatr Scand, 69 425, 1980.—A neonate severely ill with pulmonary hemorrhage improved on intravenous tolazoline. We suggest that persistent fetal circulation may complicate this disorder and that vasodilator therapy is justified when other therapeutic measures fail

KEY WORDS Newborn, pulmonary hemorrhage, tolazoline therapy

Pulmonary hypertension causing right to-left shunting of blood through the ductus arteriosus and foramen ovale occur in the neonate secondary to hypoxia caused by birth asphyxia and pulmonary parenchymal disorders (2, 4) Therapy with tolazoline, a pulmonary vasodilator, has been lifesaving in cases of hyaline membrane disease (3), meconiumaspiration and intrauterine pneumonia (2) not responding to general supportive treatment

We report a case of pulmonary hemorrhage in a neonate responding to intravenous tolazoline

CASE REPORT

A girl was born at term after a normal pregnancy The
the
heart
were
hours
and
cyanosis One hour later she appeared shocky with poor peripheral perfusion There was audible rales over both lungs and the liver was moderately enlarged *Hb* was 201 g/l, platelets 200×10⁹/l Arterial bloodgases were pH 7 09 P_{CO_2} 7 9 kPa (59 mmHg), BE −12 8, P_{O_2} 5 4 kPa (41 mmHg)

A chest radiograph demonstrated dense lungfields bilaterally She ... ...ely intubated Large amounts of hemorrhagic fluid was aspirated from the trachea during the procedure and the following hours Despite one blood transfusion the Hb fell to 186 g/l at 10 and 130 g/l at 40 hours of age She was paralyzed with pancuronium bromide and ventilated on a Bourns Infant Pressure ventilator in 100% oxygen Acidosis and hypercarbia were corrected but she remained hypoxic with arterial P_{O_2} varying between 5 and 7 5 kPa (38–57 mmHg)

minute, the systolic blood pressure was not obtainable and the liver increased considerably in size within 30 min Tolazoline (Priscoline®) was given i v in a scalp vein in a dose of 6 mg in 10 min The colour improved within

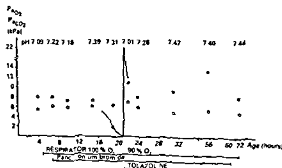


Fig 1 Blood gas values and therapy (arterial P_{O_2}, ●, arterial P_{CO_2}, O, transcutaneous P_{O_2},—) 1 kPa= 7.5 mmHg.

seconds, and 3 min after starting the infusion a transcutaneous P_{O_2} of 22 kPa (166 mmHg) was registered Tolazoline infusion at a rate of 1–2 mg/kg per hour was continued for 30 hours Ventilatory support was discontinued 16 hours later There is no evidence of permanent damage

DISCUSSION

We are not aware of other reports describing tolazoline therapy in neonatal pulmonary hemorrhage The disorder probably reflects massive pulmonary vascular congestion with rapid evolution of hemorrhagic fluid into the interstitial tissues and air spaces (1) In our patient the condition was probably a result of perinatal asphyxia as judged by the meconium staining of the amniotic fluid (6) Interstitial and alveolar oedema lead to hypoxia, hypercarbia and acidosis, conditions known to cause constriction of the pulmonary arterioles and central right-to-left shunting of blood (5)

Pulmonary hemorrhage in the neonate is usually fatal (1) We suggest that central right-to-left shunting of blood (persistent fetal circulation) may complicate the disorder Although vasodilator therapy with tolazoline is dangerous (2) a therapeutic trial is justified in the desperately ill child where all other measures have failed

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Submitted Sept 19 1979
Accepted Oct 18 1979

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Acta Pædiatr Scand 69 427–430, 1980

CASE REPORT

COLOBOMATA OF THE IRIS, CILIARY BODY AND CHOROID IN AN INFANT WITH OESOPHAGO TRACHEAL FISTULA AND CONGENITAL HEART DEFECTS AN UNKNOWN MALFORMATION COMPLEX

K LILLQUIST M WARBURG S RY ANDERSEN and I HÄGERSTRAND

From the Department of Paediatrics Odense University Hospital The Copenhagen Eye Clinic for the Mentally retarded The Institute of Eye Pathology in Copenhagen and Pathological Institute Odense University, Denmark

ABSTRACT Lillquist, K , Warburg, M , Ry Andersen, S and Hagerstrand, I (Paediatric Department, Odense University Hospital, The Copenhagen Eye Clinic for the Mentally Retarded, *The Institute of Eye Pathology* in Copenhagen and Pathological Institute, Odense University Hospital, Denmark) Colobomata of the iris, ciliary body and choroid in an infant with oesophago-tracheal fistula and congenital heart defects An unknown malformation complex Acta Paediatr Scand, 69 417, 1980.—A malformation pattern, not previously reported consisting of microphthalmia, uveal coloboma, oesophago-tracheal *fistula and congenital* heart defects is presented The phaenotypical similarity and difference from previously reported cases are discussed

KEY WORDS Congenital malformations, uveal coloboma, oesophago-tracheal fistula, eye defects

We have observed a patient with a malformation pattern which has not been described previously this included microphthalmia, bilateral uveal coloboma, oesophago tracheal fistula and heart defects A description is given of the phaenotypic similarity of the malforma tions and the difference between the present and earlier published cases is discussed

CASE REPORT

B R A 021177 1813 The patient a boy was the third child of a 27 year-old woman Children numbers 1 and 2 are healthy The parents are not consanguineous Both the mother and grandmother suffer from Recklinghausen s disease The mother has pericentric inversion of chromosome 9

There were *no infections haemorrhage or drug* in take during pregnancy Intrauterine growth retardation was suspected due to low oestriol and gonadotropin values throughout the whole of the pregnancy The birth took place at term The placenta was small 346 g and the umbilical cord contained only one artery Birth weight 2560 g and length 51 cm At birth the infant was apnoic and had irregular heart action suction and ventilation resulted in normal respiratory and heart rhythm

A chromosome study showed pericentric inversion of chromosome No 9 (karyotype 46 XY, inv (9))

Microphthalmia as well as microcornea were observed after delivery Bilateral iris coloboma were present towards six o clock and ophthalmoscopy revealed a sector shaped coloboma downwards in the choroid and in the optic papilla on both sides No cataract or haemorrhage could be observed In addition low set and curled ears were present together with right sided facial paralysis A [illegible]

[illegible] demonstrated during the subsequent catheterization and angiocardiography These were an atrio-septal defect, left sided superior vena cava and an abnormal origin of the right subclavian artery

The infant was operated on at the age of one month for the oesophago-tracheal fistula Nevertheless his condi tion deteriorated and death occurred at the age of 3½ months

Autopsy

Weight 3130 g 53 cm in length There were no abnormal findings in the respiratory tract Small scars were found in the trachea and oesophagus following the operation, no strictures There was a persistent left superior caval vein and a persistent foramen ovale *(15×15 mm) The* aortic ostium had two valves, and three openings of coro-



Fig 1 Vertical section of the one eye A malformed optic papilla and a coloboma of the choroid are seen (arrows) through the window

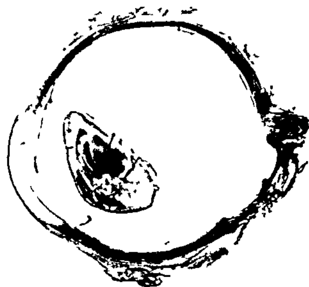


Fig 2 Vertical section of the one eye demonstrates a cataractous dislocated lens a small remnant of the iris downwards and a coloboma of the optic papilla Haematoxylin eosin (×3)

nary arteries were observed The pulmonary ostium showed one atrophic and two normal valves The right subclavian artery appeared as an aortic branch distal to the left subclavian artery and running behind the oesophagus over to the right side No malformations were found in the central nervous system or skeleton Semiserial microscopical sections showed that the right and left halves of the pons were symmetrical and normal On the other hand neither the right nor the left facial nucleus could be identified Histological examination of the other organs showed pronounced general blood congestion and pulmonary arteries of foetal type (pulmonary hypertension)

Examination of the eyes showed

Macroscopical examination Small eyes measuring 17×17×17 mm Vertical section A coloboma could be seen in the iris ciliary body choroid and optic papilla at six o clock (Fig 1)

Microscopical examination The cataractous lens was dislocated downwards and backwards (Fig 2) As usual a small remnant of the iris was seen in the area of the coloboma The disc was malformed with a process of mesenchymal tissue extending into the vitreous (Fig 3) Prolapsed dysplastic retinal tissue was seen at the papilla and in the optic nerve, further a small island of cartilage was observed in the nearby sclera possibly an abnormal differentiation from the neural crest (Fig 4)

DISCUSSION

The present patient and his mother had inversion of chromosome 9, which is a relatively common anomaly and which is usually considered harmless (2) The patient had a malformation pattern consisting of microphthalmia, bilateral coloboma of the iris, ciliary body, including the papilla, complicated cardiac malformations and on oesophago tracheal fistula

Autopsy of the brain provided no explanation of the rightsided facial paralysis and paralysis of the right vocal cord Anophthalmia, atresia of the oesophagus and oesophago tracheal fistula have been observed in a two-month old foetus which also had cebocephaly (6) A male patient with a monocular coloboma of the choroid, narrowing of the trachea, congenital heart disease hypoplasia of the acetabulae, hypospadias, posterior uretral valves and lowset ears has been reported previously (3), and finally, a number of patients have been described with microphthalmia or colobomata, congenital heart disease and various other malformations Some of these patients had malformations of the skeleton, while others had urogenital malformations and malformed ears A few have had microphthalmia or colobomata and congenital heart disease In none of the last mentioned cases was an oesophago tracheal fistula present (7) Congenital heart disease and atresia of the oesophagus, urogenital malformations and skeletal malformations together with anal atresia have been de-

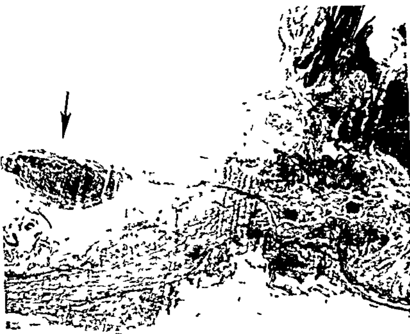


Fig 3 Coloboma of the choroid with dysplastic retina A process of mesenchymal tissue invades the vitreous from the malformed optic papilla (arrow) Masson Trichrome staining (×17)

scribed in the VATER (VACTERL) syndrome but colobomata or microphthalmia have never been reported with this malformation association (1 5 7)

Patterns of malformation which have not previously been described may be termed malformation complexes (4) The present patient has such a malformation complex We have described it in order to elicit descriptions of similar cases



Fig 4 Optic nerve with coloboma and prolapsed retina An island of cartilage may be seen in the sclera (arrow) Haematoxylin eosin (×17)



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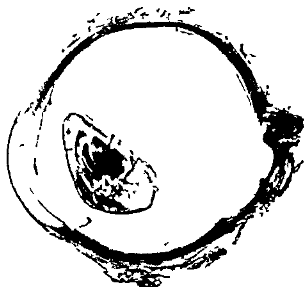


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ACKNOWLEDGEMENT

The authors would like to thank Dr Ursula Friedrich, University of Aarhus for describing the karyotype

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Submitted Nov 5, 1979
Accepted Nov 28 1979

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To Henry Kempe at his reception of the Rosén von Rosenstein Medal, May 1979

The Swedish Paediatric Association is happy to announce that it has decided to award the Rosen von Rosenstein Medal to Dr Henry Kempe, of Denver, Colorado, USA I feel very proud of having been chosen to introduce Dr Kempe

In 1964, on the occasion of the bicentenary of the publication by Nils Rosen von von Rosenstein of his textbook "Diseases of Children and Their Remedies", the first modern textbook in pediatrics in the world literature, the Swedish Paediatric Association decided to create a token of merit for the most outstanding pediatricians of our days and that it should bear the image of Rosén von Rosenstein Everybody in this audience knows Dr Kempe by his work and is also aware of his creativity, and his concern for suffering and underprivileged infants and children all over the world Certainly there is general agreement in this country that Dr Kempe in all respects meets the qualifications of being the most evident recipient of the Rosen von Rosenstein Medal

Dr Kempe s career as a physician and teacher resembles in many ways that of Nils Rosen von Rosenstein Both have made basic contributions to medical science and both have tackled practical and clinical problems of extreme importance for mankind They have both realized that in order to improve conditions it is necessary not only to educate doctors and health personnel but also to inform the public about all aspects of the issues which are under debate Both Nils Rosen von Rosenstein and Dr Kempe have been general educators, the main reason for their achievements being partly due to the considerable effort they have applied to this task

The greatest concern of Dr Rosen von Rosenstein and of Dr Kempe has been the safety of children In the now classic book 'The Battered Child' which was edited by Ray Helfer and Henry Kempe and published in 1968, the motto is a citation from Rousseau

"Let us speak less of the duties of children and more of their rights"

For Kempe the meaning of this sentence has been that children should not be rejected by their parents and that they should have access to society They should also have the right to be raised without hunger, fear, abuse, or neglect Rosen von Rosenstein devoted all his talents to saving children from poor nutrition, poor hygiene and serious and fearsome infectious diseases such as smallpox By his work he also proved that he shared Rousseau's views on the rights of children

When Dr Kempe was appointed as professor and chairman of the Department of Paediatrics at the University of Colorado School of Medicine in Denver, he was a well known virologist, who had made important scientific contributions to that field From observations which were made during his routine clinical work Dr Kempe found, that non accidental injuries were much more common among infants and small children than had hitherto been anticipated In 1961, at the Annual Meeting of the American Academy of Paediatrics he led a symposium on the various aspects of child abuse or "the battered child syndrome" as he designated the condition A clinical report which was published the following year in the Journal of the American Medical Association served as a real alarm signal In the United States as well as in many European

ANNOUNCEMENTS

THIRD INTERNATIONAL SYMPOSIUM ON ADVANCES IN PEDIATRIC RESEARCH

The Third International Symposium on Advances in Pediatric Research— Current Views on the Etiology and Pathogenesis of Insulin-dependent Diabetes Mellitus is to be held October 20–23 1980 at the Hospital for Sick Children Toronto Ontario Canada

Twenty-one invited speakers will discuss the morphological functional and metabolic changes on insulin dependent diabetes autoimmunity HLA system virus and islet-cell antibodies in the etiology of the disease insulin receptors and counter regulatory hormones epidemiological studies in various populations animal models of insulin-dependent diabetes Registration will be limited to 150 For further information please write Dr Julio M Martin Research Institute The Hospital for Sick Children 555 University Avenue Toronto Ontario Canada M5G 1X8

FIRST INTERNATIONAL SYMPOSIUM ON RECENT ADVANCES IN PRENATAL DIAGNOSIS

At the Palazzo della Cultura e dei Congressi in Bologna (Italy) on the 15th and 16th of September 1980 the First International Symposium will be held on Recent Advances in Prenatal Diagnosis The Symposium organized by the Obstetrical and Gynecological Department of the Bologna University and by the F Angelini Research Institute Rome will include a number of Round Tables on the main aspects of prenatal diagnosis Presidents of the Congress are Prof P E Polani (London) Prof C Orlandi (Bologna) Organizing Secretariat and information Assistenza Congressi Via P Palagi 21 40138 Bologna Tel 051 394882

FIRST INTERNATIONAL CONGRESS OF PEDIATRIC LABORATORY MEDICINE

The 1st International Congress of Pediatric Laboratory Medicine is to be held Oct 12–17 1980 in Jerusalem For further information write to the Secretariat 1st International Congress of Pediatric Laboratory Medicine 7 Letteris Street P O B 16271 Tel Aviv Israel Abstract forms should be submitted no later than June 1

SECOND ANNUAL MEETING OF THE EUROPEAN PAEDIATRIC RESPIRATORY SOCIETY

The 2nd Annual Meeting of the European Paediatric Respiratory Society will be held Oct 9–11 1980 in Baden bei Wien Vienna Austria Topics CNSLD in children interstitial lung disease bronchiectasis small airway disease standardization of lung function testing gas exchange Information may be obtained from the Conference Secretary Dr M H Gotz Univ Kinderklinik Wahringer Gurtel 74–76 A 1090 Vienna Austria

Acta Pædiatr Scand 69: 435–440, 1980

THE NATIONAL CENTER IN DENVER

B F STEELE

It is a great privilege and honor to be here today and receive the Nils Rosen von Rosenstein Medal on behalf of my dear friend, Professor Henry Kempe He deeply regrets his inability to be here because of ill health, and sends to you his highest regards, warm best wishes, and great appreciation of the honor you bestow upon him I am sorry you do not have the privilege of seeing and hearing him in person

When I knew I was coming here for this occasion, I took a few minutes to go to our library and learn something about Nils Rosen von Rosenstein I am a psychiatrist, not a pediatrician, and had not been acquainted with him before It was a pleasure to find that Rosenstein, your famous "father of pediatrics , and Dr Henry Kempe shared some of the same interests Both of them were practitioners, clinicians and educators, and each wrote a textbook of pediatrics Both of them were concerned with the interaction between mother and child Both of them were interested in infectious diseases, especially smallpox and immunizations Dr Kempe was very active in the work which has resulted in the recent eradication of smallpox all over the world But, it is his work on behalf of abused and neglected children for which Henry Kempe has become most well known, and it is about this subject that I would like to say a few words today

In the late 1950 s Dr Kempe became very concerned about the number of children with non accidental injuries who were seen on the pediatric wards of the University of Colorado Hospital in Denver The diagnosis of the injury was medically accurate, but the patterns of parental dysfunction relating to it were not well understood, and the causes and treatment of parental abuse were relatively unknown In 1960 he coined the phrase, "Battered Child Syndrome", and in 1962 was the chief author of a paper on this subject which drew the attention of both the medical profession and general public to the plight of these infants and children He then enlisted the aid of other pediatricians, nurses, social workers, and myself as psychiatrist, to work with him on the problem By this time he had developed the concept of the multi-disciplinary Child Protection Team as a most effective means of dealing with cases of child abuse and their management, and this type of organization has now been usefully established in many places in the United States and in other countries all over the world Active research into the causes, various manifestations and treatment of child abuse and neglect rapidly increased during the ensuing years under Dr Kempe's inspiration and direction, and became organized in 1972 into a special unit of the Department of Pediatrics under the name of "The National Center for Prevention and Treatment of Child Abuse and Neglect"

Treatment has always been the central theme around which all other aspects of our work has been organized There are several reasons for this focus on treatment First, of course, is the obvious orientation of the physician towards caring for the injured child Second, is the necessity to treat the parents so as to change and improve their ability to adequately care for their offspring Even in cases in which a child is removed from his home to protext him from *further injury*, *it is necessary* to treat the parents because sooner or later the

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Address given at Upsala Sweden, May 18, 1979 by Brandt F Steele, M D substituting for C Henry Kempe M D

countries the medical profession, social workers and the public were awakened to the startlingly high incidence of the severely battered child in our society It was also realized that a great number of maltreated children than reported go unrecognized, undetected and unprotected and that the abused child syndrome may be one of the leading causes of death in infancy and childhood.

Thanks to the devoted work of Dr Kempe and his co-workers—among whom I should like to mention his wife Ruth, who is a psychiatrist, Patricia Beezley (social worker), Christy Cutler (psychologist), Brian Fraser (lawyer), Ray Helfen (pediatrician) and Brandt Steele (psychiatrist)—all aspects of child abuse and neglect were elucidated It was proved that maltreatment of children exists not only in families which are socially deprived—people of every socio-economic, educational, religious and geographical background were found to have abused children in their custody

After having elucidated the clinical and psychosocial features of child abuse and neglect, Dr Kempe and his co-workers have turned their attention to the problem of helping battered children and their families Methods of prediction have been worked out as well as of treatment modalities Indefatigably they have also educated professionals and laymen about how to protect children and how to prevent them from being abused and neglected A great number of well-written books have been edited by Dr Kempe, in which he and his co-workers have reported the results of their own studies and given their views on how to save children from maltreatment These books are now used in many countries as a study material for health personel and social workers Some of them have also been translated from English to a number of other languages, at least two of them to Swedish

Dr Kempe is the founder of the International Society for Prevention of Child Abuse and Neglect. The first conference of the society was held in Geneva in 1976 and was attended by about 400 participants from all over the world. The congress was a great success and a second congress will be held in London in September this year

In order to encourage multidisciplinary research on the various aspects on maltreatment of children an international *ad hoc* working group under the chairmanship of Dr Kempe has started the International Journal Child Abuse & Neglect with Dr Kempe as an extremely hard-working editor-in chief The first issue of the journal appeared in 1977 Since then the journal has gradually grown in importance and the number of submitted papers is increasing rapidly

It is quite obvious that very few have had the capacity to do as much for the children of our days as has been done by Dr Kempe We all know that Dr Kempe was looking forward with great enthusiasm to attending the annual meeting of our association and to receive the Rosen von Rosenstein awards We very much regret that a sudden illness has made it impossible for him to attend and that we cannot welcome him here in Uppsala as our distinguished guest We are however, very pleased that his colleague Dr Brandt Steele has been able to come here on his behalf

May I ask you Dr Steele to forward to Dr Kempe the congratulations of the Swedish Paediatric Association

Rolf Zetterström

Acta Pædiatr Scand 69 435–440, 1980

THE NATIONAL CENTER IN DENVER

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their own early years, and are really only grown up, abused children themselves In many ways we are, therefore, treating the same problem in its early and in its later phases as we work with these children and parents

The treatment program which all of us enjoy the most is the Therapeutic Playschool It cares for children ages 2½ to 5 years old from 8 30 a m to 3 00 p m four days a week In the beginning we worked with children who had been physically abused or seriously nutritionally deprived In the last three years we have included sexually abused children and they now comprise two thirds of those we care for There are, of course, some differences in these children related to the kind of abuse they have suffered, but they share many of the same problems and can be cared for together They are children who are so seriously disturbed they would fail when they entered the usual school system at age five or six They are depressed angry, fearful, lonely distrustful, and usually unable to play well or be happy Delays in speech and motor development are common, and their relationships with adults are difficult in many different ways

We have been very pleased to find that nearly all of these children, under the guidance of our devoted and especially skilled teachers, have shown remarkable improvement Many of them in only six months or a year have shown enormous gains and are able to resume their normal developmental course, and can successfully make the transition to the regular school system The parents of the children in the playschool are also given counseling and education in child care, and helped to understand the problems of their own individual child and how best to live together more successfully Some of the children need special, individual therapy in addition to the regular school session because of the severity of some emotional or obvious difficulty It is always a great joy to see these unhappy, hurt, sad children begin to blossom and really live

We also provide group sessions and some individual therapy for older children and their parents, often oriented towards special needs of individual cases Some parents need education in the general area of child development and what to expect from their children at different ages Others need help in learning the simple mechanics of child care, and still others need help in learning methods of coping with all the usual, simple emotional problems occurring in normal parent–child interactions We often video tape sessions with these parents and review the tapes with the parents to show them the meaning of different interaction with their children and how they can be improved Some long term psychotherapy and counseling are also provided for parents by social workes and psychiatrists In many instances, we have maintained such contact for many years, partly because of the continued need, but mostly because of research interest and a desire to follow up these children to see what happens to them I have followed some families now for nearly twenty years, and have watched the children grow up to be quite healthy, and relatively normal We are, of course, aware that the "natural history" of child abuse has been changed by our involvement

Not all cases are successful We have had significant failures in trying to take care of children who were severely traumatized both physically and emotionally For instance, two children who had seen a sibling killed by repeated physical abuse, have never really been able to adjust well in life, have done poorly in school, and are now becoming juvenile delinquents This occurred despite all the help we knew how to give them Such long term care, of course, is too expensive, and there are not enough trained professionals to accomplish it We, therefore, developed what Dr Kempe called his Lay Therapy Program These were carefully selected women who have raised children of their own and who could provide on going, supportive, friendly guidance for families in trouble over long peri

child will probaby be returned to their care Also, these parents already have, or will in the future have, other children so it is necessary to help them learn better techniques of child care, how to manage their emotional problem, and how better to deal with all the social, marital, and economic problems in their lives

We were interested in doing clinical research on the causes of child abuse, on why parents had such maladaptive patterns of child care We soon found that offering sympathetic medical, social and psychological help was the most effective way of becoming acceptable to the parents to overcome their distrust and be involved in their lives enough to understand all the facets of their abnormal behavior It was in the context of treatment that the relationship developed which made research possible

Dr Kempe has always insisted that everyone who is involved in the educational work of our Center must also be caring for abused children or their parents We do a great deal of teaching of students and training workers from all over the United States, trying to give the best information we have to those who must deal with the problem Dr Kempe has a strong belief that no one should teach others how to do something unless he is actively involved in doing it himself Therefore, our treatment program is the essential basis of our educational work and our publications We must train pediatricians, social workers, psychiatrists, school teachers, lawyers, and health visitors Therefore, we have all of these kinds of professional people on our staff working in the various treatment programs actively involved with abused children and their families

The objectives in our treatment program have been primarily to find ways to help parents and children live more rewarding lives which lead to more optimal development of the children and prevent injury to them We have tried to be innovative in developing types of treatment which can be applied in as many places as possible, and in as many different kinds of organizational settings as possible, in rural areas, as well as in large cities We also realize that the cost of treatment must be kept within reasonable limits Some treatment methods we have tried and found to be effective, but they are too expensive and, therefore, cannot be practically applied in most situations For example, we had one project which we called "Circle House', involving the care of three or four families at a time Parents and children moved into a home where we provided 24-hour care and protection of the children and various forms of counseling and therapy for the adults The fathers continued to work and the mothers were helped in all forms of child care and development, as well as household economy We had hoped to rehabilitate these abused families in three months but it usually took six months or more The plan was very effective, but it was extremely expensive and was stopped after two years because continuation was impracticable Despite its effectiveness, it could not be established as a treatment method in the areas where it was most needed

We also established a Crisis Nursery where parents who were under stress and fearful of hurting their babies could bring their children at any time of day or night, and leave them in the care of the nursing staff for as long as three or four days while they received help in overcoming the crisis in the family This project demonstrated that we could greatly diminish the number of injuries occurring in troubled families on an emergency basis, even though all the fundamental problems were not solved We closed our own Crisis Nursery after several years, because the social agencies within our metropolitan area had established other nurseries modeled after our pilot project, and ours was no longer essential

We also have other on going treatment programs designed to help both the abused/neglected children and their parents or other caretakers The children are those who have suffered all the various kinds of physical injuries, medical and nutritional neglect, emotional or sexual abuse Most of the parents we see were themselves neglected or abused in

also self help groups of parents involved in sexual abuse quite similar to the Parents Anonymous Groups organized for parents who were physically abusing children In treatment, our aim is to restore self esteem, to restore a sense of privacy for the child, and help him develop the ability to say ' No ' to any further exploitation It is also necessary to change family patterns and solve marriage problems, so the child is no longer in danger

We have been aware for many years that the problems of maltreatment of children are too great and the cases too numerous to be managed by just picking up the broken pieces and trying to glue them back together, so it was inevitable that Henry Kempe, with his interest in immunization and prevention of disease, would tackle the problem of prevention of child abuse We had found that maltreatment of infants could be well understood as a disorder of attachment, a failure to establish empathic bonds between parent and child at the time of birth Therefore, it was most logical to try preventive techniques in the peri natal period In pilot studies we have found that observations of poor attachment behavior between mother and infant in the delivery room and during the first few feedings of the infant were very accurate predictors for later parenting problems Further, all cases of mal treatment of babies occurred in members of this high risk group We then found that some relatively simple services provided for these high risk families seemed to prevent serious maltreatment The families showed a striking decrease in the incidence of severe abuse or serious neglect when compared to a control group of similar high risk families who received routine, good care, but no special services It is well known that families prone to maltreatment are likely to be isolated, suspicious and highly resistant to any interference by any outside agencies, and by authorities or professionals But even in such negativistic families the event of childbirth is apparently the one time in their lives when they will accept, tolerate, and even welcome outside interest and assistance Therefore, this seems to be the best time to intervene The special interventions we use are relatively simple, and do not require starting a whole new program We use already existing personnel in a different way Nurses are taught to recognize signs of poor attachment, and to help the new mother overcome her first doubts and difficulties, supporting and encouraging her in her care of the new baby Instead of just giving the mother an appointment for a check up in the clinic in six weeks, the pediatrician becomes acquainted with mother, father and baby in the hospital, and makes contact with them every two weeks or oftener The same pediatrician is seen by the family every time they come to the outpatient clinic The public health nurse sees the mother in the hospital, and makes several home visits instead of the usual perfunctory single visit The social worker, too, makes early contact and maintains a friendly on going relationship with home visits Most importantly, a non professional Lay Health Visitor with special orientation toward problems of abusive families begins contact in the hospital and maintains it for several weeks or months, providing friendly supportive care, advice and companionship This is a valuable procedure which is quite similar, I believe, to the Health Visitor you have used for some time in Sweden In short, these families are provided with the basic lifelines which any normal, healthy family has with its relatives and professional help Instead of mounting tension and crises without ways of coping and then exploding against the children, these people now have safe ways to call for help, thus minimizing the chances of maltreatment to occur All tendencies toward abusive behavior are not eliminated, but the on going safe contacts make it possible to help when there is only a mild tendency to maltreat the child, and equilibrium can be quickly restored In those families who have not received special services, the usual instances of more serious injury have occurred and help is given with great difficulty We feel that these preventive measures

ods of time, and to help families reach proper channels for professional help when needed. This use of non-professional therapists has been demonstrated to work extremely well, and similar programs have been started in many places.

Because not all families can be kept intact, because of the necessity to put a child in protective care, or because the parent has extreme difficulty in learning how to properly care for children, we have felt it necessary to study the problems of foster care and improve them. We have developed a program of selecting better foster parents and supporting them in care, not only of the foster child, but also of the natural parents. We thereby maintain contact between natural parents and child in order to strengthen the inadequate attachment bonds, and this also provides education in parenting for the natural parents similar to that they would have if they were fortunate enough to have a good extended family. We have found that this shortens the period of foster care necessary, helps the child, and is economically feasible. In such situations we can also demonstrate much earlier the need to terminate parental rights and to place a child for adoption.

Because of the large number of children suffering from nutritional neglect and failure to thrive because of inadequate parental care, we have developed programs for detailed intensive study of such difficulties to determine the characteristics of parents who are unable to properly care for their children in this way and to understand the distorted relationship between parent and child which develops into the feeding difficulty. Therapy is provided for mothers by both pediatricians and social workers and lay therapists helping such distressed parents develop greater attachment for their children and increased ability to care for them adequately.

Most recently we have become involved in problems of sexual abuse of all kinds and in children of all ages. There has been a marked increase in the reporting of such abuse, partly due to the increased public acceptance of the problems of abuse in general and also due to the effect of the feminist movement or "Women's Lib", which has encouraged women to complain and say they will not put up with abuse any longer. Often there is a combination of both sexual and physical abuse in the very early years. Our attention is often drawn to cases through the reporting of the physical abuse; only later is the accompanying sexual abuse apparent. Most sexual abuse occurs in a family setting, and requires a great deal of work with the family. Incest of various types is our commonest problem. The parents need much counseling or therapy to overcome the disturbed interpersonal relationships in the marriage. The damage done to children, requiring care and treatment, is not only the result of the sexual event itself, but more importantly the emotional trauma of feeling exploited and used inappropriately by adults. They feel abandoned, disregarded, not cared about, not listened to. They have very low self-esteem and a great deal of shame and guilt.

The problems of sexual abuse have quite different meanings according to the age of the child, the child's psychosexual development, the kind of abuse, the duration, and the relationship between the victim and the abuser. Therefore, there is a need for therapeutic endeavors of many different kinds. We have been including the small children under age five who have been sexually abused, in our regular preschool. We also have groups of latency-age children, ages 6–11, other groups of children in puberty, around 11 to 14, adolescents 14–18, and finally, groups for young adults who were abused earlier in life. In addition to group therapy for these various ages, we have provided individual counseling and psychotherapy. There is also a necessity, in addition to treating the victims, to provide some sort of care, counseling for the perpetrators and their spouses. And, it has also been found necessary at times, to provide treatment for the siblings of the abused child. There are

also self help groups of parents involved in sexual abuse quite similar to the Parents Anonymous Groups organized for parents who were physically abusing children In treatment, our aim is to restore self esteem, to restore a sense of privacy for the child, and *help him develop* the ability to say "No" to any further exploitation It is also necessary to change family patterns and solve marriage problems, so the child is no longer in danger

We have been aware for many years that the *problems* of maltreatment of children are too great and the cases too numerous to be managed by just picking up the broken pieces *and* trying to glue them back together, so it was inevitable that Henry Kempe, with his interest in immunization and prevention of disease, would tackle the problem of prevention of child abuse We had found *that maltreatment* of infants could be well understood as a disorder of attachment, a failure to establish empathic bonds between parent and child at the time of birth Therefore, it was most logi*cal to try preventive techniques in the peri*natal period In pilot studies we have found that observations of poor attachment behavior between mother and infant in the delivery room and during the first few feedings of the infant were very accurate predictors for later parenting problems Further, all cases of mal*treatment of* babies occurred in members of this high risk group We then found that some relatively simple services provided for these high risk families, seemed to prevent serious maltreatment The families showed a striking *decrease in the incidence of severe* abuse or serious neglect when compared to a control group of similar high risk families who received routine, good care, but no special services It is well known that families prone to maltreatment are likely to be isolated, *suspi*cious, and highly resistant to any interference by *any outside agencies*, and by authorities or professionals But even in such negativistic families the event of childbirth is apparently the one time in their lives when they will accept, tolerate, and even welcome outside interest and assistance *Therefore*, this seems to be the best time to intervene The special interventions we use are relatively simple, and do not require starting a whole new program We use already existing personnel in a different way Nurses are taught *to recognize* signs of poor attachment, and to help the new mother overcome her first doubts and difficulties, *supporting* and *encouraging* her in her care of the new baby Instead of just giving the mother an appointment for a check-up in the clinic in six weeks, the pediatrician becomes acquainted with mother, father and baby in the *hospital, and makes* contact with them every two weeks or oftener The same pediatrician is seen by the family every time they come to the outpatient clinic The public health nurse sees the mother in the hospital, and makes several *home visits instead* of the usual perfunctory single visit The social worker, too, makes early contact and maintains a friendly on going relationship with home visits Most impor tantly, a non-professional Lay Health Visitor *with special orientation toward problems of* abusive families begins contact in the hospital and maintains it for several weeks or months, providing friendly supportive care, advice and companionship This is a valuable procedure *which is quite similar, I believe, to* the Health Visitor you have used for some time in Swe den In short, these families are provided with the basic lifelines which any normal, healthy family has with its relatives and professional help *Instead of mounting tension and crises* without ways of coping and then exploding against the children, these people now have safe ways to call for help, thus minimizing the chances of maltreatment to occur All tendencies toward abusive *behavior are not* eliminated, but the on going safe contacts *make it possible to help* when there is only a mild tendency to maltreat the child, and equilibrium can be quickly restored In those fami lies who have not received special services, the usual instances of more serious injury have *occurred* and *help* is given with great difficulty We feel that these preventive measures

are similar to some things which are already occurring in Sweden, and which account for your lower incidence of child abuse

After noting the success demonstrated over a period of three years in our pilot studies, we have now been offering such special services to all families living in Denver County who have babies at our hospital each year This constitutes 20% of the 3000 babies born each year in our facility All the data so far indicate very significant reduction in abuse and neglect of infants It is also cost effective

In one pilot study, we calculated that at a cost of $25000 we probably saved our State of Colorado nearly one million dollars But the actual monetary saving is insignificant compared to the enormous saving in human suffering and prevention of tragedy Not only are these babies protected from serious neglect and injury, but we prevent the formation of another generation of adults who might also abuse or neglect their offspring We hope to report further results in the near future

SERUM CHOLESTEROL IN EARLY CHILDHOOD

Familial and Nutritional Influences and the Emergence of Tracking

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ABSTRACT Boulton, T J C (Department of Paediatrics, University of Adelaide, The Adelaide Children s Hospital South Australia) Serum cholesterol in early childhood Familial and nutritional influences, and the emergence of tracking Acta Paediatr Scand, 69 441, 1980.—The influence of familial and nutritional factors on serum total cholesterol (TC) and the age at which evidence of tracking appeared, was investigated in the first 2 years The level of association between the parents' and children s TC levels increased to [illegible] fluence on the children s TC of nutritional and familial factors, the parents' TC levels and a family history of early coronary heart disease were found to be important correlates of the children s TC Low correlations occurred for nutritional factors The degree of tracking of cholesterol (the extent to which the level maintains its relative position in the distribution curve through time) as measured by the correlation coefficient of the level at time one on the level at time two, increased through infancy to r 0.40 from one to two years The association between the TC level in cord blood and later levels increased to two years (r 0.32) The results are interpreted as showing either familial influences on a child s TC level become increasingly important from the end of the first year, or that the effect of these influences is obscured during infancy by other, presumably nutritional, factors which themselves are elusive because of the inherently inadequate methodological techniques of dietary studies

KEY WORDS Cholesterol, childhood, nutrition

The differences in prevalence rates for coronary heart disease (CHD) between countries (9 13) is partly related to the differences in serum cholesterol (TC) levels (24) Nutritional factors largely account for these differences, and clear differences in TC and triglyceride (TG) levels also occur in the same population between vegetarians and people on a mixed diet (16 18) An infant s TC can certainly be altered by changes in dietary fat and cholesterol composition (11 20) Differences in activity level, body fat, and genetic factors exert smaller effects on individual differences (7 19)

Despite the evidence for the importance of dietary factors in communities cholesterol levels, within a homogeneous group of healthy families these effects may be blurred, with no correlation between diet and serum cholesterol being found (8, 23)

As hypercholesterolaemia (HC) is common and is unequivocally associated with CHD, it is of importance to know whether HC adults were HC in early life Conversely, are children with TC levels in the upper percentiles destined to become HC adults? In other words, when does tracking of cholesterol start?

This study was therefore designed to determine both the extent of familial and nutritional factors in the level of cholesterol in early child-

are similar to some things which are already occurring in Sweden, and which account for your lower incidence of child abuse

After noting the success demonstrated over a period of three years in our pilot studies, we have now been offering such special services to all families living in Denver County who have babies at our hospital each year This constitutes 20% of the 3 000 babies born each year in our facility All the data so far indicate very significant reduction in abuse and neglect of infants It is also cost-effective In one pilot study, we calculated that at a cost of $25 000 we probably saved our State of Colorado nearly one million dollars But the actual monetary saving is insignificant compared to the enormous saving in human suffering and prevention of tragedy Not only are these babies protected from serious neglect and injury, but we prevent the formation of another generation of adults who might also abuse or neglect their offspring We hope to report further results in the near future

Table 3 *Correlates for the serum cholesterol level at intervals through the first 2 years*

Months	3 to 6	(n)	6 to 12	(n)	12 to 24	(n)
Combined	0 34***	297	0 38***	274	0 40***	188
Boys	0 25**	151	0 30***	140	0 40***	96
Girls	0 45***	146	0 43**	134	0 40***	92

**$p<0\ 01$ ***$p<0\ 001$

curred between fathers and sons at one year (r 0 34, $p<0\ 01$) and at 2 years (r 0 39, $p<0\ 002$), and between mothers and sons at one (r 0 24, $p<0\ 05$) and 2 years (0 45, $p<0\ 001$) There was no correlation between the mothers' and fathers' TC levels

For the hierarchical inclusion regression, the two following data sets were used as independent variables (a) the nutritional variables, and (b) the mothers' TC (TCM), the fathers' TC (TCF) and the FHCD For (a) low levels of correlation with TC occurred at each age point studied, with none of the factors (daily intake of fat etc) being consistently selected as the most significant

Table 2 shows the variable selected first for boys and girls separately with the multiple r value achieved after all the nutritional variables had been included For the second data set (b) the boys' multiple r value was maximal at one year (0 58 $p<0\ 05$) with the mothers' TC being consistently selected first at each age point For the girls the correlates reached 0 36 at 6 months and 0 24 at 2 years, with no variable being consistently selected first (Table 2) It was noteworthy that the FHCD variable—reflecting the families' experience of premature CHD—was selected first for the girls at two age points, and second for the boys at each age apart from one year (not shown in table) When both data sets were included in one analysis the parents' TC levels were at a higher level of correlation than the nutritional variables at each age point

The correlates between the TC level in cord serum and postnatally rose from 0 20 at 3 and 0 18 at 6 months, to 0 29 ($p<0\ 001$) at one year and 0 32 ($p<0\ 001$) at 2 years The associations for LDL and HDL levels between birth and one year were 0 25 ($p<0\ 005$) and 0 31 ($p<0\ 001$) respectively

The extent of tracking as a reflection of the establishment of a regulatory system for serum cholesterol, was measured by regression analysis of the level at time point one on time point two Table 3 summarises the data and shows that tracking started during the first year, but increased in significance through the second year for the boys, reaching 0 40 ($p<0\ 001$) for the whole sample between one and 2 years For LDL the correlate was 0 35 ($p<0\ 001$), but did not reach significance for HDL

DISCUSSION

The serum cholesterol level increases rapidly in the postnatal period, and in the children comprising this study sample it continued to rise until the end of the first year, then declined to 2 years (5)

Tracking of the TC level became established during the first year, with evidence that for the girls this occurred earlier than for the boys The sample sizes were sufficiently large to suggest that this was a real difference and not a statistical artefact The increasing level of association between the TC level in cord blood and the subsequent levels suggested that extraneous (possibly nutritional) factors were confounding the expression of genetic influences and were causing a delay in the establishment of the child s regulatory system for serum cholesterol

The results also showed that the positive associations between parents' and children's serum cholesterol levels became increasingly

Table 1 *Coefficients for correlation for serum total cholesterol between parents and children*

Child s TC at	Birth	(n)	3	(n)	6	(n)	12	(n)	24	months (n)
Father s TC	0 12 n s	76	0 18*	125	0 18*	125	n s		0 25**	113
Mother s TC	0 20*	120	0 13*	190	0 14*	193	0 17*	189	0 25***	180

n s not significant $^*p<0$ 05 $^{**}p<0$ 01 $^{***}p<0$ 001

hood, whether these influences change in relative importance during the first 2 years of life, and when tracking begins

MATERIALS AND METHODS

The sample comprised a group of children randomly selected by birth order and prospectively followed until 2 years of age 391 were seen at 3 months 325 at 6 months 265 at one year and 198 at 2 years The serum cholesterol values of 127 fathers and 196 mothers were also measured and included in the analyses The methods used for blood sampling and the analysis of TC low and high density lipoprotein cholesterol (LDL HDL) have been described previously (3) The statistical analyses used comprised both simple and hierarchical inclusion regression (12) in which a multiple r value was calculated and the independent variables ranked according to their influence on the dependent variable—in this case TC

The nutritional variables used included the mean daily intakes of protein carbohydrate fat (g) and cholesterol (mg) and the mean daily food energy intake per kg body mass The methods used in the collection of these data and the actual results at each age have been reported separately (4) As the breast fed babies data could not be included because values for their daily food intakes were unknown the numbers used in the analyses for 3 months (when nearly half the babies were breast fed) were lower than the actual sample number seen

The family histories of cardiovascular disease (FHCD) were graded arbitrarily in the following manner

High risk a history of angina or myocardial infarction through two or more consecutive generations e g paternal great grandfather and paternal grandfather

Moderate risk an isolated coronary event in one or more non consecutive generations or on different sides of the family

Low risk no history of angina nor infarction but reported occurrence of hypertension stroke HC diabetes or peripheral vascular disease in one or more generations

No risk no reported history of any of the previously mentioned conditions

Although this variable was at an interval level only rather than at an ordinal level as for the other variables it was considered a valid method of introducing this factor into the analyses

RESULTS

The positive associations between the parents and children s TC levels increased in strength and significance from birth reaching r 0 25 ($p<0$ 001) at 2 years of age (Table 1) These associations were rather stronger for the boys than for the girls At 2 years the correlations were for mothers sons 0 35 ($p<0$ 001) mothers daughters 0 10 (n s) fathers sons 0 28 fathers daughters (both $p<0$ 05) For HDL only weak associations occurred and for LDL the only significant associations oc

Table 2 *Results of the hierarchical inclusion regression analysis for data sets (a) the nutritional variables and (b) the parents TC and FHCD showing the variable selected first and the multiple r value reached*

Age (months)		3 r	n		6 r	n		12 r	n		24 r	n
Set (a) Boys	Fat	0 14	106	Protein	0 14	108	Carbohydrate	0 15	132	Fat	0 13	97
Girls	Cholesterol	0 12	118	Cholesterol	0 14	109	F level insufficient			Carbohydrate	0 15	90
Set (b) Boys	TCM	0 47	22	TCM	0 51	23	TCM	0 58*	23	TCM	0 53	22
Girls	FHCD	0 26	26	FHCD	0 36	26	F level insufficient			TCF	0 24	25

$^*p<0$ 05

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Submitted April 6 1979
Accepted Jan 2 1980

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strong from the end of infancy, suggesting the emergence (or possibly derepression) of familial influences The lack of any association between the parents' own TC levels makes environmental factors unlikely The significance of the small sex differences in the level of these associations, being consistently stronger for boys' TC and LDL, is unclear but may reflect a time difference in genetic expression of cholesterol metabolism Andersen & Friis-Hansen (1) found a rather similar degree of association between the TC level of children aged from 14 to 19 months and their fathers (r 0 19) and mothers (r 0 22, $p<0$ 001 for both), and Godfrey et al (6) also found similar levels of correlation in young schoolchildren Mimura (10) found a parent child correlation of 0 26, and between sibs of 0 28, and Valkenberg et al (21) found the correlation to be higher for child mother (0 36) than for child father (0 22), as did Schaeffer et al (17) (0 36 and 0 20 respectively)

Although qualitative differences in the intake of dietary fat and fatty acids are causally associated with differences in the serum TC levels both for sub populations (14, 15) as well as for individual adults (22) and babies (11) community studies on the effect of diet on TC usually fail to find an unequivocal relationship (8) This may be accounted for by the intrinsic methodological errors involved in such nutritional studies, by the small variation in diet on a homogeneous sample and by fluctuations of individuals' TC level being of equal magnitude to the actual degree of diet induced differences (2) The factors may explain the apparent lack of significant association between the children's TC levels and dietary factors

A graduated scale of the family history of premature CHD (FHCD) in the analyses was found to be a strongly influential variable on a child's TC, being selected before the parents TC level at 3 and 6 months for the girls and after a parent's TC level at each age but one year for the boys This raises the question as to the significance of differences in serum cholesterol levels within the observably normal range in relation to CHD A separate analysis in this sample showed that those with a history of early CHD through two consecutive generations had a higher mean TC than those children with no such history

In conclusion, these results may be interpreted as showing that tracking of cholesterol levels becomes established during infancy coincident with the strengthening of the positive associations between parents and children's cholesterol levels Nutritional factors were found to exert a smaller observable influence, although the inherent limitations of nutritional analyses probably prevented their full expression

ACKNOWLEDGEMENTS

These studies were supported by the Adelaide Children s Hospital Research Foundation I also wish to thank the Director and Mr G Hill of the Dept of Chemical Pathology the Adelaide Children s Hospital for their continued help Mrs Lyndal Coote performed the dietary analyses and Mr Michael Scutter handled the data analysis

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LDL RECEPTOR STUDIES IN CHILDREN WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA (FH) MEASUREMENT OF STEROL SYNTHESIS IN BLOOD LYMPHOCYTES

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ABSTRACT *Andersen* G E. and Johansen, K B (the Neonatal Department, Rigshos-

[illegible]

FH heterozygotes was only about half of the LDL suppression in [illegible] however there was a considerable overlapping of LDL suppression in FH heterozygotes and normals Finally in one family LDL suppression was strictly normal in FH heterozygotes who thus appear to have a different type of FH not involving an impaired LDL receptor function

KEY WORDS Hypercholesterolemia, lipoproteins, cholesterol, sterols, lymphocytes

Heterozygous FH is one of the commonest inborn errors of metabolism (2) FH is an autosomal dominantly inherited disease with a 2–3 fold increase in serum T-C and/or LDL C either vertically in three generations or in a family with xanthomatosis Clinically planar and tuberous xanthomas and xanthelasmas may develop Premature and severe coronary heart disease in male heterozygotes occur already in early or mid adult life (7) Elevated serum LDL C appears to be a better discriminant between FH patients and normals than elevated serum T-C alone (13), but even so serum T C and LDL-C values overlap in unaffected and FH heterozygotes (14) In certain cases there is a need for supplementary diagnostic tools, and it has therefore been speculated (1, 3) that the combined measurement of serum LDL C *and* of the number of cellular LDL receptors might allow a more accurate diagnosis, since the primary genetic defect in FH involves the gene encoding a high affinity LDL cell surface receptor which binds and internalise serum LDL Normal fibroblasts, arterial smooth muscle cells, lymphoblasts and lymphocytes have been shown to derive the major part of their cholesterol from circulating LDL via these receptors (5, 9, 10) Furthermore it has been shown that the binding of LDL to LDL receptors elicits a suppression of intracellular sterol synthesis by inhibiting the rate limiting enzyme HMG CoA reductase (4) This means that the synthesis of cellular cholesterol is subject to a feedback regulation mediated specifically by LDL binding to the LDL receptors Fibroblasts and lymphocytes from FH heterozygotes have been shown to express only about one *half the normal* number of LDL receptors measured by

Abbreviations FH=familial hypercholesterolemia, TG= [illegible] =very low [illegible] LDL=low [illegible] g/ml), HDL=high density lipoproteins (density 1 063–1.215 g/ml), LPDS=lipoprotein-deficient serum (density >1.215 g/ml), P/S=polyunsaturated/saturated fatty acids, HMG CoA=3-hydroxy 3-methylglutaryl coenzyme A.

Statistical analysis of the data was performed by calculating the means and standard deviations and using Student's *t* test for differences

RESULTS

Since the number of LDL receptors on lymphocytes is relatively low immediately after their removal from the bloodstream where they are exposed to LDL (3) we first incubated the lymphocytes in a medium devoid of LDL (i e LPDS) to increase the number of LDL receptors and sterol synthesis (maximal stimulation) and hence increase the binding of added LDL in order to discriminate a reduced number of LDL receptors and reduced LDL suppression of sterol synthesis in FH heterozygotes better from normal Furthermore maximal suppression was achieved by the addition of 7 ketocholesterol which crosses the lymphocyte membrane independent of the LDL receptors and suppresses sterol synthesis by direct inhibition of the rate limiting enzyme HMG CoA reductase (12) In Table 1 the values for incorporation of [2 ¹⁴C]acetate into lymphocyte sterols are presented after maximal stimulation maximal suppression and LDL suppression expressed in dpm/μg lymphocyte protein Furthermore LDL suppression is expressed as the percentage

$$\frac{(\text{Maximal stimulation} - \text{LDL suppression}) \times 100}{\text{maximal stimulation} - \text{maximal suppression}}$$

In five families 1 4 5 6 and 7 (Table 1) it is seen that the LDL suppression is clearly reduced in FH heterozygotes compared with normals In these five families a clear pattern emerges indicating that the expected reduced number of LDL receptors is in fact reflected by the reduced LDL suppression of sterol synthesis

In families 9 and 10 there is a considerable overlapping of LDL suppression values in FH heterozygotes and normals In these families the diagnosis FH would not have been possible without knowing the serum LDL C values and the family history of hypercholesterolemia This is particularly true for 9 III 6 in whom an LDL suppression of 41 4 was found indicating that this boy has FH although his serum T C and LDL C values are completely normal This family 9 was investigated three times with at least one month intervals and practically the same LDL suppression values were found each time

Families 3 and 8 are puzzling In both families the mothers (3 III 3 and 8 III 7) have LDL suppression values in the normal range whereas their sons (3 IV 1 and 8 IV 3) have clearly reduced LDL suppression values Even in these families LDL suppression studies were repeated (twice) with at least one month interval and almost the same values were found

In family 2 the LDL suppression values are seen to be in the normal range in FH heterozygotes thus indicating that in the FH heterozygotes nothing is wrong with the function of LDL receptors in suppressing sterol synthesis This family is the only one of the ten families in which such a pattern emerged

When the LDL suppression values were pooled in all FH heterozygotes a mean ±1 S D of 49 9±17 1% was found (5 III 3 was not included) This is significantly lower ($p <$ 0 01) than a mean ±1 S D of 67 1±11 6% in normals In family 5 the heterozygous mother (5 III 3) died from her second myocardial infarction in March 1979 Her LDL suppression had been shown to be clearly reduced (25 4%) when we measured it using a slightly different technique 2 months before she died In families 4 and 6 the fathers (4 II 5 and 6 III 1) had died 35 and 31 years old of a myocardial infarction No LDL suppression studies were available From Table 1 it is also seen that sterol synthesis rises to a wide range of values after maximal stimulation both in normals and FH heterozygotes

DISCUSSION

The finding of a threefold variation in sterol synthesis values after maximal stimulation

29 80 874

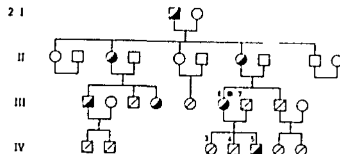


Fig 1 Kindred 2 (The 9 other pedigrees can be requested from the authors (G E A)) □ ○ Not sampled (deceased or abroad) ⊠ ⊘ normal ◢ ◑ type II • xanthomas present

the binding and degradation of ^{125}I-labeled LDL after incubation under culture conditions which elicit a maximal rate of receptor synthesis (3) Other methods for LDL receptor estimation have been described (5) We here decided to measure the LDL suppression of lymphocyte sterol synthesis to test the hypothesis that lymphocytes from FH heterozygotes with one half the normal number of LDL receptors would be expected to have one half the normal LDL suppression of sterol synthesis

SUBJECTS

48 members from 10 families with FH were selected for the study One of the FH families (family 2) is presented in Fig 1 The pedigrees for the 9 other families can be requested from the authors (G E A) The family members here investigated are all marked by an arabic numeral in Fig 1 They were all on a National Institute of Health Type II diet with a P/S ratio of 1 0–1 5 including the unaffected spouses and siblings since they lived together and were sharing the same household None of the FH heterozygotes were treated with drugs except 8 II 3 and 9 I 4 who for the last 5 and 3 years resp had taken clofibrate 0 5–1 5 g a day Venous blood was drawn under sterile conditions after a fast of at least 12 hours even in the youngest children

METHODS

Serum lipoproteins from the family members were separated by ultracentrifugation (8) LDL (density 1 019–1 063 g/ml) and LPDS (density >1 215 g/ml) were prepared from EDTA plasma of healthy subjects by differential ultracentrifugation The protein content of the lipoprotein fractions was measured by the method of Lowry et al (15) using bovine serum albumin as a standard The lipids of the lipoproteins were measured enzymatically as described earlier (11)

Peripheral blood lymphocytes were isolated under sterile conditions as described by Ho et al (9) Each washed cell pellet containing 15–22×10^6 mononuclear cells from 15 ml of venous blood was resuspended in 15 ml medium A (RPMI 1640 medium plus 2 mM L glutamine) with penicillin 100 IU/ml and streptomycin 100 µg/ml containing 10% (vol/vol) human LPDS (final concentration 3 mg LPDS protein/ml) The cell suspension was divided into 6 portions of 2 ml (each containing 2–4×10^6 cells) which were transferred to 6 2 5 ml plastic culture tubes Cell viability was assessed by the criterion of erythrosin B exclusion Differential counts showed that 89–97% of the mononuclear cells were lymphocytes and that 3–11% were monocytes All 6 tubes were incubated at 37°C in a humidified incubator (5% CO_2 in air) Two of the 6 tubes were incubated for 68 hours (maximal stimulation) Another 2 of the 6 tubes were incubated for 53 hours before the addition of 7 ketocholesterol in ethanol (final concentration 5 µg/ml) (maximal suppression) The final 2 of the 6 tubes were also incubated for 53 hours before the

incubations (68 hours) [2 ^{14}C]acetate (specific activity 4 878 mCi/mmol) was added to all 6 tubes (final concentration 2 5 mmol/l) The 6 tubes were then further incubated for exactly 4 hours and the incubation was terminated by transferring the cell suspensions to 15 ml

(6) The nonsaponifiable lipids were extracted with three 4 ml portions of *n* hexane and centrifuged for 5 min at 2000 rpm between each extraction The pooled hexane extracts were backwashed with 2 ml of a 0 1 N NaOH

out in a Betaszint BF 5000 liquid scintillation counter for 10 min Quench correction was made by channels ratio The nonsaponifiable lipids were quantitatively isolated in toto No separation into individual sterols was carried out since earlier studies (10) and our own results (unpublished data) have demonstrated that there is a linear correlation between the incorporation of [2 ^{14}C]acetate into cholesterol and nonsaponifiable lipids

Before saponification 200 µl of each of the 6 cell suspensions were transferred to 400 µl microfuge tubes Each cell pellet was isolated by centrifugation for 12 min at 4000 rpm and washed with 150 µl 0 9% NaCl three times The final cell pellet was resuspended in 200 µl 0 1 N NaOH and left overnight at 37°C before the protein content was determined by the Lowry method (15) The [2 ^{14}C]acetate→[^{14}C]sterols was expressed in dpm/µg cellular protein (mean of two determinations) The day to day variation was assessed by the inclusion of lymphocytes from the same healthy subject on 8 occasions when the family members were studied LDL suppression was 73 5±6 08% in the normal subject

(Table 1) is in accordance with the results of Bilheimer et al (3) who found that the number of LDL receptors also varied about threefold within the group of normals and within the group of FH heterozygotes after maximal stimulation

In the healthy adult a mean LDL suppression of sterol synthesis of 73 5% was found This value is comparable to the 75% LDL suppression found by Ho et al (10) and about 82% reported by Postle et al (16) In 17 normal subjects from the ten FH families a mean LDL suppression of 67 1% was found which is significantly higher than a mean of 49 9% in the 30 FH heterozygotes In the individual families however, LDL suppression studies clearly discriminated FH heterozygotes from normals in only five families (1, 4, 5, 6 and 7) These five families resemble the one large family described by Bilheimer et al (3) in which ¹²⁵I LDL degradation studies allowed a clear distinction between FH heterozygotes and normals In four of the ten families (3 8 9 and 10) the picture was much more unclear with a considerable overlapping of LDL suppression values in FH heterozygotes and normals In these four families LDL suppression studies alone would not have made possible the diagnosis FH These four families thus illustrate the difficulties foreseen by Bilheimer et al (3) who speculated that when turning from the one large family mentioned above to

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are actually seen to discriminate FH heterozygotes from normals much better than LDL suppression

Family 2 is an exception and the most interesting of the ten families The heterozygous mother (2II16) and her heterozygous son (2IV5) have strictly normal LDL suppression values which means that their LDL receptors seem to function normally in suppressing sterol synthesis In these two individuals we hypothesize that their elevated serum LDL C might be the result of a strict overproduction of LDL but a normal LDL catabolism, i e a normal LDL receptor pathway This hypothesis is currently being investigated

In the present study there was no significant correlation between serum LDL C levels and LDL suppression values in either FH heterozygotes or normal subjects or both, thus confirming an earlier study (3) which also was unable to demonstrate any correlation between plasma LDL C values and the number of LDL receptors This again means that although the genetic defect in FH seems to be in the regulation of the number and function of LDL receptors, the level of serum LDL is also controlled by a different mechanism which involves the synthesis of LDL and which is still poorly understood

ACKNOWLEDGEMENTS

This study was supported by grants from the Danish

the preparation of the manuscript

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Table 1. *Serum lipid and lipoprotein-cholesterol values and lymphocyte sterol synthesis in 48 members of 10 FH-families*

Kindred	Diagnosis	Age (y)	T C (mmol/l)	VLDL C (mmol/l)	LDL C (mmol/l)	HDL C (mmol/l)	TG (mmol/l)	Maximal stimulation (dpm/μg)	Maximal suppression (dpm/μg)	LDL suppression (dpm/μg)	LDL suppression (%)
1 IV 1	FH	29	13 21	1 53	10 22	1 46	3 21	6 31	1 37	4 52	36 2
1 IV 2	Normal	26	5 28	0 20	4 18	0 90	0 82	5 63	1 05	2 49	68 6
1 V 1	FH	6	10 01	0 42	8 27	1 32	1 32	5 69	1 36	4 19	34 6
2 III 6	FH	43	8 12	0 36	6 77	0 99	1 14	6 64	1 09	2 31	78 0
2 III 7	Normal	43	6 94	0 47	5 19	1 28	2 00	7 02	1 54	2 00	91 6
2 IV 3	Normal	19	5 10	0 27	3 60	1 23	1 00	5 98	0 83	2 05	76 3
2 IV 4	Normal	18	3 89	0 15	2 78	0 96	0 96	5 97	1 11	2 10	79 6
2 IV 5	FH[a]	13	6 38	0 14	4 89	1 35	0 62	7 33	0 81	1 76	85 4
3 III 2	Normal	37	4 92	0 17	3 40	1 35	0 95	2 84	1 15	1 69	68 0
3 III 3	FH	32	8 53	0 07	7 22	1 24	0 63	5 01	1 00	2 50	62 6
3 IV 1	FH	14	7 49	0 18	6 14	1 17	0 80	4 36	0 74	2 95	39 0
3 IV 2	Normal	12	4 00	0 10	2 47	1 43	0 73	6 09	1 00	2 84	63 9
4 III 4	FH	27	7 44	0 25	6 41	0 78	0 90	5 29	1 02	2 61	62 8
4 IV 3	FH	9	7 44	0 10	5 93	1 41	0 70	7 10	1 54	3 55	63 8
4 II 6	Normal	35	4 98	0 16	2 92	1 90	0 82	8 39	3 45	5 51	58 3
4 III 6	FH	16	8 94	0 84	7 07	1 03	2 73	5 73	2 33	4 35	40 6
4 III 7	FH	13	7 89	0 19	6 82	0 88	0 91	6 37	2 67	5 54	22 4
5 III 3	FH	34	8 67	0 91	6 91	0 85	2 30				25 4[b]
5 III 2	Normal	41	6 51	0 98	4 56	0 97	3 23	8 14	1 68	3 35	74 1
5 IV 1	FH[a]	5	5 97	0 31	4 66	1 00	1 08	6 25	1 37	4 15	43 0
5 IV 2	FH	3	6 33	0 29	5 23	0 81	1 13	5 78	1 30	2 96	62 9
6 IV 1	FH	22	8 26	0 10	7 15	1 01	0 64	4 66	1 22	3 84	23 8
6 IV 2	FH	18	7 22	0 12	5 92	1 18	0 74	6 83	1 39	5 34	27 4
7 I 7	FH	34	14 00	0 90	12 32	0 78	1 89	3 40	1 71	2 67	43 2
7 I 8	Normal	32	5 59	0 22	4 08	1 29	0 80	3 91	0 83	1 52	77 6
7 II 9	FH	9	9 07	0 17	7 85	1 05	0 61	4 12	0 86	2 46	50 9
7 II 10	FH	5	9 98	0 22	8 43	1 33	0 71	5 82	1 05	3 30	52 8
7 II 11	FH	3	7 39	0 22	6 28	0 89	0 78	6 66	1 13	3 23	62 0
7 I 9	FH	31	7 21	0 45	6 13	0 63	1 54	5 31	0 71	4 43	19 1
7 I 10	Normal	30	3 85	0 25	2 35	1 25	0 85	9 72	1 27	3 66	71 7
7 II 13	Normal	10	4 59	0 28	2 30	2 01	1 20	7 23	2 29	4 34	58 5
7 II 14	FH	5	7 67	0 28	6 20	1 19	1 07	9 05	1 54	7 06	26 5
8 II 3	FH	54	10 13	0 31	8 75	1 07	1 24	3 86	1 16	2 37	55 2
8 III 7	FH	31	6 56	0 15	5 35	1 06	0 82	3 38	1 52	2 01	73 7
8 III 8	Normal	31	4 86	0 15	3 50	1 21	0 82	9 87	1 47	4 77	60 7
8 IV 2	FH	6	7 79	0 19	6 58	1 02	0 86	4 09	1 18	2 07	69 4
8 IV 3	FH	2	7 43	0 21	6 47	0 75	1 14	9 05	1 26	6 10	37 9
9 I 4	FH	59	9 57	0 19	8 04	1 34	1 10	6 87	1 32	3 63	58 4
9 II 5	FH	32	7 80	0 26	6 20	1 34	1 03	6 18	0 87	3 09	58 2
9 II 6	Normal	30	6 50	0 62	4 66	1 22	2 09	6 42	0 80	3 31	55 3
9 III 4	FH	8	6 59	0 25	5 20	1 14	0 97	4 78	0 77	3 05	43 1
9 III 5	FH	3	6 40	0 42	5 07	0 91	1 46	11 63	1 50	6 66	49 1
9 III 6	Normal	10	4 21	0 17	2 66	1 38	0 70	8 22	1 10	5 27	41 4
10 II 2	FH	23	10 41	0 12	8 63	1 66	0 80	5 86	1 02	3 24	54 1
10 II 3	Normal	25	5 03	0 17	3 42	1 44	0 76	9 09	1 18	4 57	57 1
10 III 1	Normal	8	4 01	0 33	2 38	1 30	0 70	6 31	1 39	2 72	73 0
10 III 2	Normal	7	3 57	0 08	2 24	1 25	0 53	8 26	1 06	3 56	65 3
10 III 3	FH	4	6 46	0 16	4 72	1 58	0 52	4 39	1 16	2 46	59 8

[b] See text

Acta Pædiatr Scand 69 453–455, 1980

THE FATTY ACID COMPOSITION OF SERUM LOW DENSITY LIPOPROTEIN- AND LYMPHOCYTE CHOLESTEROL ESTERS IN CHILDREN WITH HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

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KEY WORDS Hypercholesterolemia, lipoproteins, cholesterol, cholesterol esters, fatty acids, lymphocytes

In FH a reduced fractional catabolic rate (FCR) of apo LDL is a typical finding (3, 9) Furthermore in FH there is a reduced number of cellular LDL receptors which specifically bind, internalise and degradate circulating LDL as described by Brown & Goldstein (5) So far one study by Thompson et al (13) has indicated that the fatty acid composition of LDL might influence LDL catabolism as well. To further investigate this hypothesis we measured the fatty acid composition of serum LDL- and lymphocyte cholesterol esters in children with heterozygous FH and in their normolipemic siblings

SUBJECTS

29 children (age 5–18 years) from 10 FH families were selected for the study 19 of the children had heterozygous FH and 10 siblings were normal The diagnosis of FH was based upon a three vertical transmission of elevated (>95 percentile value for normal Danish children (8)) serum T-C and LDL-C on more than three occasions In the normal siblings serum lipids and lipoproteins were

venous blood was drawn ...

METHODS

Serum lipoproteins were separated by ultracentrifugation (6) The lipids of the lipoproteins were measured enzymatically as described earlier (8) FC was measured enzymatically omitting however the cholesterol esterase from the analysis CE was calculated as the difference between T C and FC Peripheral blood lymphocytes were isolated under sterile conditions as described by Ho et al (7) The lymphocytes were washed twice in 0 9% NaCl The fatty acid composition of serum LDL-CE and lymphocyte CE was measured after extraction of the lipids with chloroform and methanol (4) and the lipid extract was stored at −20°C, until CE was isolated by thin layer chromatography using *n* hexane–diethyl ether–glacial acetic acid—70 30 1 (v/v) as solvent CE fatty acids

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Abbreviations FH=familial hypercholesterolemia, T-C= total cholesterol FC=free cholesterol, CE=cholesterol ester, LDL=low density lipoproteins (density 1 006–1 063 g/ml), P/S=polyunsaturated/saturated fatty acids, FCR=fractional catabolic rate

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Submitted Oct 18, 1979
Accepted Dec 15, 1979

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ACKNOWLEDGEMENTS

This study was supported by grants from the Danish Heart Foundation and the Dagmar Marshall Fund Britta Frank Larsen and Inger Jeppesen provided excellent technical assistance We thank Gitte Femerling Thurmer for the preparation of the manuscript

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Submitted Nov 30, 1979
Accepted Dec 15, 1979

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Table 1 *Fatty acid composition of serum LDL-CE (mean percentage ±1 S D)*

	FH heterozygotes (n=19)	Normals (n=10)	p
14 0	1 19±0 58	1 08±0 49	NS
16 0	12 04±1 85	12 26±1 51	NS
16 1	2 85±1 14	2 99±0 70	NS
18 0	1 11±0 27	1 17±0 18	NS
18 1	16 81±3 41	17 30±2 61	NS
18 2	58 78±7 64	58 96±4 80	NS
20 2	0 45±1 97	–	
20 4	6 75±1 61	6 24±1 70	NS

were transesterified immediately with methanol and hydrochloride as described by Stoffel et al [12] Hydroquinone (0 2 mg/ml) was added during the transesterification procedure in order to prevent oxidation of the fatty acids The fatty acid composition was determined by gas liquid chromatography on a 10% DLGS column on Chromosorb W AW DMCS 80/100 Because of lack of material lymphocyte CE fatty acids were only determined in 27 children

Statistical analysis of the data was performed by calculating the means and standard deviations and using Student's t test for differences

RESULTS

In normal subjects the mean ±1 S D serum LDL FC concentration was 1 07±0 27 mmol/l and the serum LDL CE concentration was 2 55±0 98 mmol/l In FH heterozygotes the serum LDL FC was 2 04±0 47 mmol/l which is significantly higher than normal (p<0 001) and serum LDL CE was 4 91±1 26 mmol/l which is also significantly higher than normal (p<0 001) The FC/EC molar ratio in FH heterozygotes was 0 42 which is not different from 0 44 in normals

In Tables 1 and 2 the fatty acid compositions of serum LDL CE and lymphocyte CE are presented No differences were found between FH heterozygotes and normals

Table 2 *Fatty acid composition of lymphocyte CE (mean percentage ±1 S D)*

	FH heterozygotes (n=16)	Normals (n=11)	p
12 0	1 51±3 28	3 00±3 51	NS
14 0	12 38±7 78	19 42±11 81	NS
16 0	26 99±12 52	21 34±5 04	NS
16 1	–	0 22±0 72	
18 0	14 05±7 77	12 22±6 65	NS
18 1	5 37±2 89	7 22±4 49	NS
18 2	9 23±8 54	11 96±5 32	NS
18 3	7 57±7 76	5 57±6 03	NS
20 0	6 14±5 88	6 44±5 08	NS
20 1	2 07±6 84	1 75±3 06	NS
20 2	14 66±12 15	10 84±8 48	NS

DISCUSSION

Our finding of an abnormally high content of FC and CE in serum LDL from FH heterozygotes confirms the results of Bagnall (2) and Shattil et al (10) who like ourselves found an almost equal increase in serum LDL FC and serum LDL CE

The fatty acid composition of serum LDL CE in FH heterozygotes and normal subjects (both on a diet with a P/S ratio of 1 0–1 5) was somewhere in between the plasma T CE fatty acid composition in healthy subjects on a regular diet reported by Allard et al (1) and the HDL CE fatty acid composition in healthy subjects on a polyunsaturated diet (P/S ratio 4 0) found by Shepherd et al (11) Our findings were very similar to the ones reported for fatty acid composition of plasma LDL CE in healthy adults by Thompson et al (13)

We did not find any difference between the serum LDL CE fatty acid compositions in FH heterozygotes and normals This indicates that the fatty acid composition of serum LDL CE has little if anything to do with the reduced fractional catabolic rate of apo LDL typically found in FH patients (9 13) This is interesting since one study seems to indicate that the fatty acid composition of plasma LDL CE in healthy adults may be correlated with LDL catabolism (13) Neither did we find any difference between the lymphocyte CE fatty acid compositions in FH heterozygotes and normals which means that cholesterol esterification in lymphocytes from FH heterozygotes seems to be normal

Acta Pædiatr Scand 69 457–465, 1980

CEREBRAL BLOOD FLOW AND EXCHANGE OF OXYGEN, GLUCOSE KETONE BODIES, LACTATE, PYRUVATE AND AMINO ACIDS IN ANESTHETIZED CHILDREN

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ABSTRACT Settergren, G , Lindblad, B S and Persson, B (Department of Paediatrics, Karolinska Institutet and the Unit of Paediatric Anesthesiology, St Goran s Hospital, Stockholm, Sweden) Cerebral blood flow and exchange of oxygen glucose, ketone bodies, lactate, pyruvate and amino acids in anesthetized children Acta Paediatr Scand, 69 457, 1980.—Cerebral blood flow (CBF) and cerebral av differences of oxygen, glucose, 3-hydroxybutyrate, acetoacetate, lactate, pyruvate and amino acids were measured in anaesthetized children before elective surgery in order to study possible age-dependent variations CBF was measured in 70 children, aged 11 days to 15 years Cerebral av differences were studied in approximately 50% of the subjects Mean values were CBF 0 65 ml × g $^{-1}$ × min $^{-1}$, cerebral exchange in nmoles × g $^{-1}$ × min $^{-1}$ oxygen 1348, glucose 248, acetoacetate 12,3 hydroxybutyrate 34 (uptake), lactate-48, pyruvate-8 (release) No net exchange of amino acids was found with the exception of histidine (uptake) Neither CBF nor the cerebral exchange of oxygen and circulating substrates showed any correlation to age within the group Compared with adults anesthetized by the same technique (barbiturate induction, nitrous oxide-oxygen relaxant) the children had a slightly higher mean CBF, while the cerebral uptake of oxygen and glucose were equal to values in adults The cerebral uptake of ketone bodies was higher in children than reported values in adults investigated in the awake state after comparable periods of fasting

KEY WORDS Cerebral blood flow, cerebral metabolism, oxygen, glucose, lactate, pyruvate acetoacetate, 3-hydroxybutyrate, amino acids infants, children

Determinations of cerebral blood flow and metabolism in children have been performed in only a limited number of studies In children with a variety of cerebral diseases the values for cerebral blood flow (CBF) and oxygen uptake by the brain ($CMRO_2$) tended to decrease with the severity of the cerebral disease (9) An analysis by Kety in 1956 of published data revealed a significant and parallel decline of CBF DMRO$_2$ and the density of cortical neurones with increasing age (12) This relationship was partly confirmed by Kennedy & Sokoloff who studied 9 healthy children 3–11 years old (11) The average values for CBF (1 06 ml×g $^{-1}$×min $^{-1}$) and $CMRO_2$ (2 308 nmoles × g $^{-1}$ × min $^{-1}$) were almost twice as high as those reported in adults Similar high values for CBF (0 90 ml × g^{-1} × min^{-1}) and $CMRO_2$ 2587 nmoles×g $^{-1}$×min $^{-1}$) were found in a group of 10 normal children with a mean age of 11 months (18) In a recent study of 19 newborn children with respiratory distress syndrome and with a mean gestational age of 33 weeks, the CBF values were surprisingly low (mean 0 31 ml×g $^{-1}$×min^{-1}) (16) One explanation for this finding was that a number of these infants suffered from arterial hypotension and impaired autoregulation of cerebral blood flow When, however, the hypotensive patients were excluded, the remaining group still had a comparatively low CBF (mean value 0 40 ml × g $^{-1}$ × min $^{-1}$) Statistical analysis of the data published by Kennedy et al (11) and Lou et al (16) reveals no correlation between age and cerebral blood flow within these two groups of children The investigations cited

Dedicated to professor Rolf Zetterstrom on his 60th birthday

Table 1 *Arterial values for oxygen tension, carbon dioxide tension, pH, base excess and blood erythrocyte volume fraction together with rectal temperature and length of fasting*

	Mean	S E	Range	Correlation with age *n*	*r*	*p*
Pa_{O_2} kPa	16 8	0 4	8 5–32 0	70	0 38	<0 001
Pa_{CO_2} kPa	5 2	0 1	3 3–8 1	70	0 04	n s
pH_a	7 35	0 01	7 20–7 47	70	0 45	<0 001
Base excess mmol/l	−4 1	0 4	15 0–+1 5	70	0 46	<0 001
B EVF%	37	0 4	23–43	57	0 60	<0 001
Rectal temp °C	36 6	0 1	34 0–38 0	70	0 36	<0 01
Duration of fasting hours	10	2 9	0 0–20 0	59	0 39	<0 01

CBF was 0 55 ml × g^{-1} × min^{-1} The 7 children who were hypercapnic (Pa_{CO_2} range 6 1–8 1 kPa) had a mean CBF of 0 73 × g^{-1} × min^{-1} However, no significant correlation between CBF and Pa_{CO_2} was observed for the group as a whole (r=0 22, n=70), as shown in Fig 2 CBF was not significantly correlated with age, neither in the group as a whole (r=−0 17) nor in the normocapnic children (r=−0 22) as shown in Fig 3

Mean values, S E , range and correlation with age of oxygen tension (Pa_{O_2}) carbon dioxide tension (Pa_{CO_2}), pH base excess and B EVF in arterial blood drawn during the CBF determination and values for rectal temperature and length of fasting are shown in Table 1 A positive significant correlation with age was observed for all these variables except for Pa_{CO_2} The mean oxygen tension (P_{O_2}) in the internal jugular vein was 6 1 kPa (S E 0 2) No correlation with age was observed CBF was not correlated to Pa_{O_2}, pH_a base excess, temperature or B EVF Analysis of partial correlations furthermore confirmed that the variation in Pa_{CO_2}, Pa_{O_2}, base excess, temperature and B EVF had no influence on the finding that CBF and age were uncorrelated

Arterial concentrations, cerebral av differences and cerebral exchange of oxygen and circulating substrates

The arterial concentrations, mean S E and correlation with age of oxygen, glucose, lactate, pyruvate, acetoacetate and 3 hydroxybutyrate in whole blood and the arterial plas-

Table 2 *Arterial concentrations of oxygen glucose lactate, pyruvate acetoacetate, 3 hydroxybutyrate free fatty acids and glycerol*

	Arterial concentration (mmol/l) Mean	S E	Correlation with age *n*	*r*	*p*
Oxygen	7 47	0 11	65	0 56	<0 001
Glucose*	5 27	0 36	42	−0 26	<0 05
Lactate	1 13	0 07	41	− 25	n s
Pyruvate	0 104	0 000	34	0 41	<0 01
Acetoacetate*	0 273	0 040	41	−0 27	<0 05
3-Hydroxybutyrate*	0 890	0 140	42	−0 33	<0 02
FFA	1 28	0 08	26	−0 46	<0 01
Glycerol	0 21	0 02	26	−0 30	n s

* Calculated from determinations of plasma concentrations and B EVF

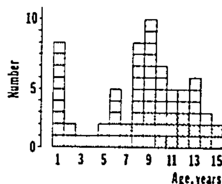


Fig 1 Age distribution of the 70 children in whom CBF was determined ☐ indicates individuals in whom the AV difference of oxygen, glucose, lactate, 3 hydroxybutyrate and amino acids in plasma were determined ☐ indicates individuals in whom the same analyses were performed except for the amino acids

above (9, 11, 16, 18) were all performed on children in the awake state

The aim of the present study was to determine if age-dependent variations in CBF, $CMRO_2$ and cerebral exchange of circulating substrates could be demonstrated in children during a standardized form of balanced anesthesia Results from 70 children between 11 days and 14 years of age and without history or evidence of cerebral disease are presented Included are previously published results from 9 infants (21)

MATERIAL

Seventy one children (59 boys and 12 girls age range 3 weeks to 14 years) were studied during general anesthesia before elective surgery The investigation was approved by the Ethical Committee Karolinska Institutet Stockholm and the parents had given their consent Seventy children had a normal development with no history or evidence of cerebral disorder while one boy suffered from Down s syndrome The results from this child have only been used for a methodological comparison between whole blood and plasma concentrations of glucose and ketone bodies The age distribution of the 70 normal children are given in Fig 1 The surgical operations performed were inguinal (cryptorchidism hernia hydrocele) in 43 operations on the extremities or genital organs in 18 laparotomies in 9 children, while one child had a thoracotomy

METHODS

The studies were performed after premedication and during a standardized form of general anesthesia The drugs used were morphine and atropine for premedication (8), thiopentone for induction (given in incremental doses until the eyelid reflex disappeared (3–5 mg/kg b w)) followed by nitrous oxide oxygen for maintenance of anesthesia At the start of the CBF measurement the nitrous oxide concentration was changed from 75 to 50% (21) Pancuronium 0 1 mg/kg b w was given as relaxant before intubation Catheters were introduced percutaneously into preferably the left radial artery and the left internal jugular vein Care was taken not to include individuals with a high oxyhaemoglobin saturation in the internal jugular vein indicating abnormalities of the venous anatomy (14,15) Rectal temperature was monitored CBF was measured using a minor modification of the Kety Schmidt technique (21) The time between induction and the determination of CBF was 20–40 minutes Blood gases (pH, P_{O_2}, P_{CO_2} and base excess) blood erythrocyte volume fraction (B EVF) and the concentrations of oxygen glucose, lactate pyruvate acetoacetate 3 hydroxybutyrate glycerol and free fatty acids (FFA) in arterial and cerebral venous blood and/or plasma were determined as described elsewhere (21) Whole blood concentrations of glucose acetoacetate and 3 hydroxybutyrate were calculated from determinations of plasma concentrations and B EVF using the formula of Dillon (6)

Six av differences from each of 7 children of glucose

to study the variation beteween and within individuals

The free amino acids of whole blood and plasma were determined on a Bio Cal 200 (Munich Germany) automatic amino acid analyser *ad modum* Spackman et al (23) Plasma samples were deproteinized by crystalline sulphosalicylic acid or by picric acid when both plasma and whole blood samples were analysed Whole blood samples were deproteinized after haemolysis by freezing and thawing the samples twice Cystine was determined as cysteic acid and methionine as methionine sulphone after oxidation (24) The samples of whole blood were treated with performic acid to completely oxidize glutathione which was then eluted at the extreme front of the chromatogram (15) Lithium buffers were used (2) The mean reproducibility for the determination of amino acids was 4 9% (S E 0 5) ranging from 1 2% (glutamine) to 9 4% (cystine)

CBF was studied in all children the other variables a shown in Fig 1

RESULTS

Cerebral blood flow

Mean CBF was 0 65 ml×g $^{-1}$ ×min $^{-1}$, (S E (0 02) Despite our intention to measure CBF in normocapnia (PA_{CO_2} 4 7–6 0 kPa) this was achieved only in 45 children who had a mean CBF of 0 68 ml × g $^{-1}$ × min $^{-1}$ Eighteen children were investigated during hypocapnia (Pa_{CO_2} range 3 3–4 6 kPa) In this group mean

Table 4 *Arterial amino acid concentration in plasma and whole blood together with the correlation coefficients (r) and levels of significance (p) for the relationship between positive plasma av differences greater than 5% of the sum of the arterial and venous concentrations and their respective arterial concentrations in plasma*

	Arterial level μmol/l						Correlation between av differences of amino acids and their respective arterial concentration		
	Plasma			Whole blood					
	Mean	S E	*n*	Mean	S E	*n*	*r*	*p*	*n*
Ornithine	72	12	18	–	–	–	0 95	<0 001	10
Arginine	45	5	21	25	2	10	0 95	<0 001	8
Histidine	70	4	28	65	3	10	0 68	<0 01	12
Cystine	71	8	27	36	3	8	0 76	<0 01	11
Lysine	215	19	28	161	13	10	0 87	<	8
Phenylalanine	40	3	29	34	2	8	0 79	<0 02	
Isoleucine	67	6	27	45	5	8	0 83	<0 001	11
Valine	201	17	27	162	15	8	0 88	<0 001	9
Leucine	109	9	29	95	10	8	0 81	<0 001	12
Methionine	25	2	24	8	1	8	0 48	n s	6
Tyrosine	38	2	29	38	3	8	0 62	<0 05	9
Aspartic acid	14	2	23	80	10	8	0 94	<0 001	10
Glutamic acid	78	9	27	166	8	6	0 50	<0 05	12
Serine	91	13	16	–	–	–	0 94	<0 001	7
Alanine	153	8	29	173	12	8	0 89	<0 01	6
Glycine	150	9	29	249	18	8	0 75	<0 01	10
Proline	105	7	19	114	9	7	0 49	n s	7
Threonine	89	9	25	77	8	8	0 96	<0 001	9
Glutamine	506	49	19	–	–	–	0 97	<0 001	7
Taurine	41	4	29	175	9	7	0 61	<0 02	12
Urea	5 748	489	17	–	–	–	0 77	<0 05	6
Asparagine	39	5	17	–	–	–	0 82	<0 02	7

droxybutyrate (r=0 82, p<0 001) The cerebral release of lactate was correlated with that of pyruvate (r=0 49, p<0 01) The cerebral release of lactate was also correlated with the cerebral uptake of glucose (r=0 33, p<0 02) A weak correlation was found between measured cerebral uptake of oxygen and the calculated uptake of oxygen needed for complete oxidation of glucose, 3-hydroxybutyrate and acetoacetate minus the release of lactate and pyruvate (r=0 29, p<0 05)

Arterial concentrations (mean, S E) in plasma and whole blood of 21 free amino acids and urea are given in Table 4 The concentrations of arginine, isoleucine and methionine were *significantly higher* (p<0 01) (*paired t-test*) in plasma than in whole blood and significantly lower (p<0 01) for lysine, aspartic acid, glutamic acid, alanine, glycine, proline and taurine Methionine concentration in arterial plasma was positively correlated with age (r=0 70, p<0 001) while a spartic acid (r≈–0 65, P<0 001) and cystine levels (r≈–0 53, p<0 01) showed a negative correlation with age The arterial plasma concentration of alanine was negatively correlated to both the arterial concentration of acetoacetate (r≈–0 61, <0 001) and 3 hydroxybutyrate (r≈–0 54, p<0 001) No correlation was observed between the arterial plasma concentrations of alanine and glucose A significant mean av difference in plasma of amino acids was found for histidine only (10 μmoles/l, p<0 01) Despite the fact that a significant mean av difference was found only for one amino acid, a number of individual av differences of amino acids were of such a magnitude that they probably were not due to the error of determination The mean reproducibility of the amino acid determinations was 5% All positive av differences in plasma exceeding 5% of the sum of arterial and venous

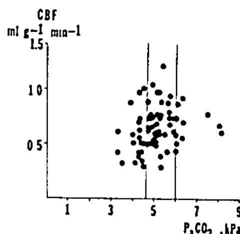


Fig 2 Relation between CBF and P_{aCO_2} Normocapnic range indicated by vertical lines

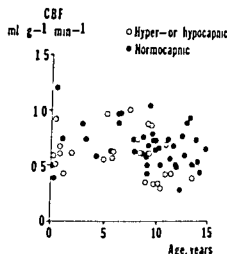


Fig 3 Relation between CBF and age in normocapnic children (●) and in hyper or hypocapnic children (○)

ma concentrations of FFA and glycerol are shown in Table 2 Positive correlations were found between the arterial concentration of FFA and glycerol ($r=0\ 53$, $p<0\ 01$) and between FFA and 3-hydroxybutyrate ($r=0\ 64$, $p<0\ 001$) Analysis of variance confirmed our earlier finding (21) that whole-blood concentrations calculated from plasma concentrations and B-EVF values involved an overestimation of the 3-hydroxybutyrate concentration ($p<0\ 05$) and also an underestimation of the glucose concentration ($p<0\ 05$) It also demonstrated that the differences between calculated and measured values were systematically greater in some children, i e a significant difference in this respect between subjects was found for glucose, acetoacetate and 3-hydroxybutyrate ($p<0\ 001$) The statistical analysis also demonstrated that the precision of mean values based on six AV differences were not significantly better than those based on three determinations

The av difference and the net cerebral exchange (mean S E) of oxygen, glucose, lactate, pyruvate, acetoacetate and 3 hydroxybutyrate are shown in Table 3 The av difference of oxygen was positively correlated with age ($r=0\ 31$, $p=0\ 01$) and the net release of lactate was negatively correlated with age ($r=-0\ 27$) ($p<0\ 05$) No other correlations with age were found The cerebral uptake of acetoacetate was positively correlated with the arterial concentration ($r=0\ 61$, $p<0\ 001$) and the same correlation was found for 3 hy-

Table 3 *Cerebral av difference and net cerebral exchange of oxygen glucose lactate pyruvate, acetoacetate and 3-hydroxybutyrate*

The individual values for the av difference are based on 3–6 paired blood samples drawn during the CBF measurement

	Cerebral av difference (mmol/l)		Net cerebral exchange (nmol g⁻¹ min⁻¹)		
	Mean	S E	Mean	S E	n
Oxygen	2 17	0 09	1 348	56	65
Glucose	0 36	0 03	248	22	42
Lactate	−0 07	0 01	−48	8	41
Pyruvate	−0 01	0 00	−8	2	34
Acetoacetate	0 018	0 002	12	2	41
3 Hydroxybutyrate	0 049	0 009	34	6	42

lated from plasma determinations and B EVF values, since determinations in *whole blood* were available only in less than 10% of the children Analysis of variance confirmed our earlier finding (21) that this could be done with adequate accuracy, though it involved a small underestimation of glucose and a small overestimation of the 3 hydroxybutyrate concentration The difference between beasured and calculated blood concentration—though small—was more pronounced in some children This significant difference between subjects indicates that the formula of Dillon suited *some of the children* better than others This is probably explained by an interindividual difference in the concentration of water in plasma and erythrocytes, since the formula of Dillon is based on mean values of the water content

As expected, the concentrations of plasma FFA and ketone bodies were higher in the younger than in the older children despite their shorter period of fasting The positive correlation between the arterial concentrations of FFA and both glycerol and 3 hydroxybutyrate together with the negative correlation between age and the arterial concentrations of FFA and 3 hydroxybutyrate may be interpreted as signs of an enhanced rate of lipolysis lipid mobilization and oxidation of FFA in the younger as compared with the older children The present observation of an inverse correlation between the concentrations of 3-hydroxybutyrate and alanine and between acetoacetate and alanine would support the concept of an intimate relationship between fatty acid oxidation–ketogenesis and gluconeogenesis (4) It could also be in agreement with the suggestion that the release of alanine from muscle is regulated by the blood level of ketone bodies (2)

The arterial glucose concentration tended to decrease with increasing age, probably due to the technique of preventing clotting of the intravenous line used for anesthesia This line was kept open by an infusion of an isotonic solution containing 1% glucose The infusion was given as an ordinary drip, and the amount of glucose × kg b w.$^{-1}$ × time^{-1} was presuably higher in the younger children.

The ratio between the mean av difference of glucose and oxygen was identical with the expected value of 1 to 6 The significance of this relation may be questioned since the *correlation between measured uptake of oxygen* and the calculated uptake of oxygen needed for complete oxidation of glucose, acetoacetate and 3 hydroxybutyrate minus the cerebral release of lactate and pyruvate was poor (r= 0 29) *We have no precise explanation for* this discrepancy, though it could indicate that the measurements were not performed during steady state conditions due to metabolic *changes induced by anesthesia*

The finding in the present study of a difference between whole blood and plasma concentration of a number of amino acids is in agreement with earlier observations (7)

Experimental studies in animals using radioactive amino acids as tracer substances have shown that the rate of cerebral influx and efflux of amino acids is approximately the same, indicating a balance between cerebral protein synthesis and catabolism (19) The observation in the present study of virtually no net mean av differences in plasma is thus an expected *finding The number of individual av* differences greater than the error of determination and the high degree of correlation between these positive av differences and their respective arterial concentrations indicate *that cerebral uptake of amino acids* could occur intermittently and that the arterial concentration is one regulating factor

The mean CMR_{O_2} and the cerebral uptake of glucose in the present study were identical *with values found in adults* anesthetized by a similar technique (1) Furthermore, there was no age-dependent variation in CMR_{O_2} or cerebral glucose uptake within our group of children Since glucose is the principal source *of energy for the brain* it was of interest to relate estimated total cerebral uptake of glucose to reported values for total glucose production rate in infants and children (3) By

concentrations were correlated to their respective arterial concentrations. For 17 out of 21 amino acids a significant positive correlation was found ($p<0.02$) The correlation coefficients for the individual amino acids are given in Table 4 The same calculation was performed with the negative av differences. For no amino acid was a significant correlation found between the arterial concentration and the negative av difference.

Whole blood concentrations of amino acids were determined in 9 subjects No significant mean av difference was demonstrated

DISCUSSION

Cerebral blood flow

The CBF values showed a large variation, though with a fairly normal distribution (Fig 2) Investigations in awake children involve various degrees of apprehension and anxiety, which might markedly influence cerebral blood flow and cerebral metabolism, thus possibly explaining the reported high values for CBF, CMR_{O_2} and cerebral glucose uptake (11, 18) Anesthesia, however, introduces another type of variation, that of the depth of anesthesia Thiopentone induces a 5 to 10 min period of anesthesia Due to the much longer time interval between induction and CBF measurement, the effect of thiopentone had probably no influence on the results Nitrous oxide-oxygen relaxant is a very superficial type of anesthesia Due to individual variation in sensitivity to morphine and nitrous oxide, some children may either have been in the excitement phase or in the phase of early surgical anesthesia during the CBF measurement This is a probable explanation for the large variation of CBF in the present study

The statistical evaluation of the results indicates that the variation in Pa_{O_2}, B-EVF, body temperature, pH_a and base excess had no influence on the variation of CBF, though individual values of these variables were outside the normal range

We have previously reported significant changes in the cerebral av difference of oxygen following changes in the arterial carbon dioxide tension, indicating that CBF is regulated by Pa_{CO_2} in the same way and to the same extent in children as in adults (20) Therefore, the lack of correlation between Pa_{CO_2} and CBF ($r=0.22$) was unexpected In the present study only small, but insignificant differences in mean CBF values between the hypo-, normo- and hypercapnic groups of children were observed The explanation for this lack of correlation between Pa_{CO_2} and CBF is probably the interindividual variation in CBF This interpretation is supported by experimental data from anesthetized dogs (25) When the first CBF determination in each animal in that study, performed during normoxia, is correlated to the corresponding carbon dioxide tension, no significant correlation is found ($r=0.37$)

The results of earlier investigations (11, 18) as well as the present data indicate that children have higher mean CBF values than adults However, no age-dependent variation of CBF was found in the children studied by Kennedy (11) or in the present study The premature infants investigated by Lou et al (16) were a heterogeneous group due to the variation in cerebral perfusion pressure In the five normotensive infants in that study, who were neither asphyxiated at birth nor hypoxic during the investigation, CBF tended to be positively correlated to age ($r=0.57$) This is an interesting observation in view of the finding by Diemer of increasing cerebral capillary density during this period of life (5)

In conclusion, available data suggest that CBF is comparatively low in the premature infant and that CBF values during childhood are higher than during adulthood

Arterial concentrations cerebral AV differences and cerebral exchange of oxygen and circulating substrates

The blood concentrations of glucose, acetoacetate and 3-hydroxybutyrate were calcu

tone bodies lactate, pyruvate and amino acids in infants *Acta Paediatr Scand*, *65* 343, 1976

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Submitted Oct 30, 1979
Accepted Jan 16, 1980

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multiplying estimated brain weight and measured cerebral glucose uptake in different age groups it was found that in the youngest group (mean age 6 months) the cerebral glucose consumption accounted for 60% of the total glucose production This figure decreased with age to 50% at a mean age of 4 years and to 40% at a mean age of 8 years The cerebral uptake of ketone bodies was higher than values reported in awake adults after an overnight fast (10) When our results were compared with those obtained in 10 normal adults who were studied awake and after an overnight fast (17), Small, but significant differences (Mann-Whitney u-test, $p<0.01$) in arterial levels were found Arterial pH, oxygen concentration and B-EVF were lower in the children, while Pa_{O_2}, lactate and pyruvate were higher The cerebral exchange values differed however only in two respects $CMRO_2$ was lower in the children, 1 340 as compared with 1 675 nmoles $\times g^{-1} \times min^{-1}$ $^{0}p<0.01$), while the uptake of 3-hydroxybutyrate was higher, 35 vis-a-vis 5 nmoles $\times g^{-1} min^{-1}$ ($p<0.01$) The difference in $CMRO_2$ between the two groups was likely an effect of anesthesia The higher uptake of 3-hydroxybutyrate in the children indicates that this substrate has greater importance for the growing brain

ACKNOWLEDGEMENT

This study was supported by grants from the Swedish Medical Research Council (projects 19X 3787 19P 4106 19X 2583) and the Tielman Fund for Paediatric Research

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Acta Pædiatr Scand 69 467–470, 1980

LUNG VOLUMES AND PULMONARY GAS EXCHANGE AFTER COARCTECTOMY

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A [illegible] Eriksson B O and Bake B (Department of Paediatric Surgery, [illegible] assessed during rest and exercise up to the maximal level [illegible] 28 years, eleven years after surgery for coarctation of the aorta Compared to the normal Swedish standards, the lung volumes indicated a restrictive impairment of lung function However, the age range in this series of patients is outside the age range of reference values for both children and adults When the effect of age on the lung volumes was allowed for they were found to be less restrictive Pulmonary gas exchange was normal at rest and during maximal exercise Thus, the aortic malformation and the thoracotomy did not leed to any marked impairment of lung function

KEY WORDS Coarctectomy, exercise studies, lung volumes, maximal oxygen uptake, pulmonary gas exchange

The introduction of coarctectomy drastically improved the prognosis for patients with coarctation of the aorta However, in contrast to the immediate excellent results after surgery, the results of long term follow up have proved less favourable Thus many patients have a persistent hypertension (6 12) An increased cardiovascular mortality has also been reported (11) This study is part of a more extensive study on the long term results after coarctectomy and concerns lung function in adult life in patients operated upon during childhood The main aim of this study was to elucidate whether we could detect impaired lung function at rest or during exercise as a late manifestation of the malformation of the aorta and its haemodynamic consequences and/or of the thoracototomy

SUBJECTS

Out of 96 children operated upon for coarctation of the aorta during the years 1958–74 at the Children s Hospital in Goteborg those who had no other known significant cardiovascular malformation were selected for this study Patients with a persistent ductus arteriosus at operation and/or bicuspid aortic valves without obstruction were not excluded Only those males who had passed 16 years of age at the time of the study and for whom an interval of at least five years had elapsed since surgery were chosen In all 25 men fulfilled these criteria Nineteen of these 25 men volunteered for the study Their mean age at operation was 10 years (range 6–14) and their present age was on average 21 years (range 16–28) Thus on average 11 years (range 6–18) had elapsed since surgery At the follow up study one patient had a slight mitral regurgitation and another patient had a mild combined mitral valve disease However their cardiovascular malformations were considered small and these two patients were also included in the study All patients were subjected to an end to-end anastomosis and no serious complication was registered during the postoperative period At the time of this study all the men were free from subjective symptoms Thirteen of the 19 patients were smokers or ex smokers

Abbreviations TLC total lung capacity l BTPS FRC functional residual capacity l BTPS RV residual volume l BTPS VC vital capacity l BTPS FEV_1 forced expiratory volume in one second l BTPS FEV% forced expiratory volume in per cent of vital capacity ΔN_2 slope of alveolar plateau of the single breath nitrogen method % N_2/l V_E total ventilation l/min BTPS VD dead space l BTPS VT tidal volume l BTPS R respiratory quotient V_{O_2} oxygen uptake l/min STPD $P_{a_{CO_2}}$ arterial carbon dioxide tension kPa $P_{a_{O_2}}$ arterial oxygen tension kPa $P_{A_{O_2}}$ alveolar oxygen tension kPa

Table 2 *Gas exchange*

Mean values with S D for some variables of *ventilation*, *intrapulmonary* gas exchange and acid base balance at rest and during exercise in 19 men operated for coarctation of the aorta during childhood Maximal exercise averaged 197 W (range 150–250 W)

	Rest	Submax I 50 W	Submax II 100 W	Submax III 150 W	Maximal exercise
Heart rate beats/min	69±9	103±15	137±16	173±14	191±7
Respiratory rate r/min	19±4	24±6	29+8	40±13	53±9
V_E l/min BTPS	8 4±2 6	26 1±5 3	40 0±6 6	66 3±12 4	104 8±21 9
R	0 86±0 08	0 86±0 06	0 91+0 06	0 98±0 07	1 09±0 11
V_{O_2} l/min STPD	0 28±0 05	0 96±0 14	1 50±0 16	2 17±0 10	2 77±0 34
V_E/V_{O_2}	30 3±5 6	27 1±3 2	26 7±3 8	30 7±5 3	39 1±5 4
VD/VT %	28±9	21±12	14±9	14±8	13±8
P_{aCO_2} kPa	4 7±0 5	4 7±1 0	4 6±0 4	4 2±0 7	3 6±0 4
P_{aO_2} kPa	13 1±1 0	13 1±0 8	12 7+1 0	12 5±1 0	12 7±1 0
P_{AO_2}-P_{aO_2} kPa	1 4±0 8	1 4±0 9	2 1±0 7	3 1±0 8	3 7±0 8
pH	7 41±0 04	7 40±0 02	7 38+0 03	7 35±0 03	7 30±0 05
Blood lactate mmol/l	0 9±0 3	1 2±1 2	2 7±1 2	3 4±2 1	10 3±1 9

creased pulmonary arterial pressure and/or an increased pulmonary blood flow However, this mechanism can hardly be valid for the Fallot cases or for the operated coarctation patients It may thus be argued that a congenital heart malformation per se might lead to a restrictive pulmonary impairment

When a group of patients are compared with a normal material it is important that the latter covers the age range of the patients The combined material of Berglund et al (4) and Grimby & Soderholm (10) which is the standard generally used in Sweden may therefore not be adequate for the present patients (16–28 years of age) When we compare our data with Swedish standards for children and young people reported by Engstrom et al (range 7–15 years) (8) and Solymar et al (range 7–18 years) (18) we find quite different results The values for the coarctation patients are then similar to or even higher than the predicted values (Table 3)

In the study of Berglund et al (4) the effect of age on VC was elucidated by combining the adult reference values with corresponding reference values for children Thus, they found an increase of VC up to the age of about 25 years and a gradual decrease thereafter We tested our values of VC against their predictions for the age range 7–70 years and found the average VC to be 88 per cent of predicted normal In fact all but three fell within ±2 S D , in spite of the high proportion of smokers When the mean values for age and height for the Fallot patients of Bjarke (5) were used they too appeared less restrictive Thus it is clearly important to use a normal standard which is adequate for adolescents and young adults

The high proportion of smokers and ex-

Table 3 *Relative lung volumes*

Spirometric values for 19 men operated upon for coarctation of the aorta during childhood in percent of predicted values according to Berglund et al (4) and Grimby & Soderholm (10) Engstrom et al (8), Solymar et al (18) and Bates et al (3)

	Berglund et al and Grimby & Soderholm	Engstrom et al	Solymar et al	Bates et al
TLC l BTPS	82±13	112±18	105±17	84±14
FRC l BTPS	93±23	166±37	127±28	87±20
RV l BTPS	81±33	119±47	140±54	76±30
VC l BTPS	83±12	109±15	99±15	88±14
FEV%	104± 6	–	101± 6	–

PROCEDURE AND METHODS

All subjects were hospitalized during the study, which also included other investigations (to be published) After a careful medical examination a chest X ray was performed Lung volumes were determined using a body plethysmograph (7), and a Bernstein spirometer was used for dynamic spirometry The single breath nitrogen method (13) was used in seventeen of the nineteen patients to determine the slope of the alveolar plateau (ΔN_2) an index of distribution of ventilation (9)

In a preliminary exercise test, designed to determine the maximal oxygen uptake, the subjects exercised on a bicycle ergometer for six minutes at at least three submaximal loads (50, 100 and 150 W) before the maximal one Maximal oxygen uptake was defined as the oxygen uptake at the highest work load they could perform for at least 3–4 minutes and until exhaustion Further criteria for inclusion were a respiratory quotient (R)>1 0 a ventilatory quotient (V_I/V_{O_2})>30 and a blood lactate concentration >8 mmol/l (1)

One or two days later a teflon cannula was introduced into the right brachial artery After 30 minutes rest in the supine position expired air was collected for at least 10 minutes for determination of ventilation and oxygen uptake Arterial blood samples for blood gases and lactate were collected simultaneously The subjects then performed graded leg exercise in the sitting position with the same work loads as in the preliminary test During the last two minutes of each work period expired air and arterial blood samples were collected simultaneously

Oxygen uptake was determined using the Douglas bag technique The volumes of the bags were measured with a gas meter The micro Scholander technique was used for gas analyses (16) Duplicate determinations were made on each bag and the coefficient of variation of the gas analyses was less than 2% Arterial blood gases were determined with a blood gas analyser (Radiometer BMS 3) Blood lactate concentration was analyzed with an enzymatic method (17) The physiological dead space was calculated using the Bohr formula and the alveolar oxygen tension from the alveolar gas equation assuming the arterial P_{CO_2} to be equal to the mean alveolar P_{CO_2}

RESULTS

The results of the spirometric determinations are given in Table 1 Lung volumes and FEV_1 were all below the predicted normal values (range 81–93%), whereas FEV% was normal in all subjects (range 78–93) ΔN_2 was above predicted normal values in six subjects (range 1 5–2 0), five of the thirteen smokers or ex-smokers and one of the four nonsmokers had an increased ΔN_2

The results of the pulmonary ventilation and gas exchange studies at rest and during exercise are given in Table 2 During maximal exercise the oxygen uptake was on average 2 77 l/min (range 2 40–3 61) The mean values during maximal exercise were heart rate 191 beats/min, respiratory rate 53 breaths/min, R 1 09 and blood lactate concentration 10 3 mmol/l, i e fulfilling the previously mentioned criteria for maximal work P_{aO_2} showed very small change from rest to maximal exercise (Table 2) Total and dead space ventilation arterial CO_2 tension, pH and blood lactate concentration also showed normal behaviour from rest up to maximal exercise (Table 2)

Table 1 *Ventilatory function*

Mean values with S D for some spirometric variables in 19 men operated upon for coarctation of the aorta during

	Estimated value	Estimated value in per cent of predicted value
TLC, l BTPS	6 0±1 2	82±13
FRC, l BTPS	3 3±0 8	93±23
RV, l BTPS	1 4±0 6	81±33
VC, l BTPS	4 6±0 7	83±12
FEV_1, l BTPS	4 1±0 6	88±12
FEV%	87±5	104± 6
ΔN_2 %N_2/l	1 4±0 4	195±62

DISCUSSION

Compared to the normal values for the Swedish adult population according to Berglund et al (4) and Grimby & Soderholm (10), as well as an American standard according to Bates et al (3), the patients showed decreased mean values for VC, TLC, RV and FRC, i e a restrictive lung impairment, eleven years after coarctectomy (Table 3) This is in accordance with the findings after total correction of Fallot's Tetralogy (5) However, the restrictive lung impairment in our patients was not so marked as in that study Similar findings have also been reported in atrial septal defect (2), in mitral stenosis (2) and in heart failure (15) The restrictive lung impairment in the latter disorders has been attributed to an in-

Acta Pædiatr Scand 69, 471–474, 1980

SPINAL CORD DAMAGE: A RARE COMPLICATION OF PURULENT MENINGITIS

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KEY WORDS Spinal cord, meningitis, cardiorespiratory arrest

Spinal cord damage and quadriplegia are very rare complications of acute purulent meningitis The purpose of this article is to present 3 such cases and to review the literature

CASE REPORTS

Case 1 R C a previously healthy 3 years and 4 months old Caucasian boy was admitted to a local hospital after 1 day of high fever vomiting and listlessness Lumbar puncture revealed purulent cerebrospinal fluid (CSF) with 950×10^6/l polymorphonuclear white cells, and *Hemophilus influenzae* bacilli were later identified on culture Eight hours after admission he had cardiorespiratory arrest and after this he required artificial ventilation On treatment with antibiotics his condition gradually improved and after 2 weeks he was beginning to talk and understand simple orders As repeated attempts to take him off the respirator failed he was transferred to the Vancouver General Hospital

On examination the child was conscious and responding Flaccid paresis of the arms was found with no demonstrable tendon reflexes The legs were spastic with brisk tendon reflexes sustained ankle clonus and extensor plantar responses There was no distinct sensory level but painful stimuli were felt more in the arms than in the legs He had no bladder control and the voiding pattern was that of spastic bladder

Electromyography (EMG) of left triceps and deltoid and of both biceps brachii muscles showed a reduced pattern and poor recruitment of motor unit action potentials an excess of polyphasic potentials and also fibrillation potentials in the left deltoid and right biceps indicating partial denervation presumably owing to myelopathy EMG of left extensor digitorum communis pectoralis major and sternomastoid showed no significant abnormality, and conduction in motor and sensory fibres of the right median nerve was normal

After 6 months it became possible to wean him off the respirator, but it took a full year until recurrent hypercapnia ceased The function of the upper limbs improved gradually, but they remained hypotonic and hyporeflexic The legs remained completely paralyzed and spastic Electrical testing 9 months after the acute disease showed improvement but remained suggestive of myelopathy

Case 2 S S, a 3½ year-old Caucasian girl, was admitted to a local hospital after 3 days of fever, lethargy and vomiting Lumbar puncture shortly after admission demonstrated cloudy CSF with 5.6×10^9/l white blood cells (94% polymorphonuclear), glucose level was 0.28 mmol/l, while blood glucose concentration was 5.7 mmol/l *Neisseria meningitidis* was cultured from this fluid

Three hours after admission the girl had a third cardiorespiratory arrest, and the right pupil was noted to be dilated After resuscitation she had to be maintained on the respirator During the following week she became alert and responsive, but still had no spontaneous respirations and was transferred to the Vancouver General Hospital

Physical examination then showed a slightly drowsy girl who did respond to environmental stimuli She moved her lips to talk but was hard to understand Optic fundi were normal apart from venous congestion The cranial nerves were normal except for partial involvement of the right third nerve, with ptosis and weakness of superior rectus muscle, and weakness of both sternomastoid muscles There was flaccid quadriplegia below the upper cervical level No tendon reflexes could be elicited Complete sensory loss was found from C1 downwards, but there were reflex withdrawal movements of shoulders,

smokers may partly explain the disturbed ΔN_2-values At least middle aged smokers frequently have abnormal ΔN_2 (14) We have no obvious explanation for the increased ΔN_2 in one non smoking subject, but the age range of the reference material (21–60 years) is again probably not adequate

Regardless of whether or not a restrictive lung impairment was present, normal values for ventilation and pulmonary gas exchange were found This was so both at rest and during submaximal and maximal exercise There was thus no obvious impairment of pulmonary function

It may be concluded that neither the coarctation of the aorta nor the thoracotomy had any marked influence on lung development or on pulmonary function Thus, in this respect patients are healthy eleven years after coarctectomy

ACKNOWLEDGEMENTS

This study was supported by grants from the Samariten Foundation for Paediatric Research the Swedish Association against Heart and Chest Diseases and the University of Goteborg

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Submitted Sept 26 1979
Accepted Jan 16 1980

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(7 10) In both these cases as well as in our 2 surviving patients the clinical signs included quadriplegia, sensory loss and lack of sphincter control All these signs gradually subsided after the acute onset though convalescence was protracted and not always complete In each of our 3 cases as well as in the case reported by De Sousa et al (7) there was a period of apnea No cardiorespiratory problem was mentioned in the case described by Gotshall (10)

There are several possible mechanisms for spinal cord damage in acute bacterial meningitis One is the actual involvement of the cord parenchyma in the infectious process with possible abscess formation We did not find any such description in the literature and in 2 of our patients it was proved not to be the case by myelography or autopsy

A second possible mechanism is vascular either ischemic or embolic The anterior three quarters of the spinal cord are supplied by the anterior spinal artery via the penetrating branches These branches act as end arteries without anastomosis and when they are occluded there follows myelomalacia of the segments supplied (24) On the other hand, the posterior part of the spinal cord is supplied by the 2 posterior spinal arteries with a rich network of anastomoses between them and is very resistant to any vascular accident The vulnerability of the anterior spinal artery to arteriosclerotic and luetic obliteration (24) and to emboli (26) has been well described in the past Narrowing and even obliteration of medium sized arteries in the brain has been shown by angiography during acute bacterial meningitis (9 13) where arteritis and phlebitis of cerebral vessels may have led to infarcts and hemorrhages of the brain We could find no reports of angiographic studies of the spinal cord during meningitis but as mentioned above the anterior spinal artery in the lowest segment of the cord of our last patient was occluded by thrombus In the higher segments the arteries were patent but contracted as in spasm

During systemic arterial hypotension blood flow in the spinal cord is self regulated, as shown in monkeys by Kobrine et al (15) In spite of this, spinal stroke has been described in the zone supplied by the anterior spinal artery following transient cardiorespiratory arrest (4, 23) As all our 3 patients and one mentioned in the literature had such an event, this might have been the cause of the myelopathy Transient ischemia might explain the widespread destructive process noted in the autopsy of the newborn (Case 3)

There are 2 less likely mechanisms for spinal cord injury during meningitis The first is transient paraplegia resulting from a hematoma caused by lumbar puncture (11, 16) This complication has been reported in patients with impaired coagulation which is, of course, a frequent concomitant of overwhelming sepsis The other possibility is myelopathy secondary to intramuscular (possibly intraarterial) injection of penicillin, also described in children in the past (3, 5)

Though extremely rare, spinal cord damage should be kept in mind in severe cases of meningitis especially in patients whose course of the disease involves cardiorespiratory arrest

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hands and feet on pinprick There was no control of bowels or bladder

To exclude the possibility of compression of the upper cervical cord by herniation of the cerebellar tonsils myelogram vertebral angiogram and pneumoencephalogram were performed in the second month after transfer All were found normal Six weeks after admission a gradual improvement began and the girl started to use the upper extremities to some extent The level of anesthesia also began to descend and was replaced by a band of hyperesthesia On reassessment after 6 months she was found to be completely alert The arms were moderately hypotonic and weak with diminished tendon reflexes and the legs were spastic with brisk reflexes Bilateral sustained ankle clonus and extensor plantar responses were elicited There was no longer any demonstrable sensory deficit and she had regained control of her bowel movements but was still incontinent of urine with reflex neurogenic bladder of the spastic type

She was last seen 2½ years after the acute illness She had mild dorsal levoscoliosis and tense spinal extensor muscles Her mental development was normal The cranial nerves appeared intact The upper limbs were hypotonic yet showed bilateral brisk tendon reflexes and moderate resistance to passive supination There was fisting of the hands on crawling especially on the left Abdominal and anal skin responses remained absent The lower limbs were spastic with brisk tendon reflexes and extensor plantar responses but she was able to walk cautiously with scissoring and hyperextension of the knees She still lacked complete bladder control but was no longer incontinent of stool

Vital capacity was only about 0 5 litres (expected 0 96) and the peak flow rate was 86 litres/minute (expected 123) An electroencephalogram was normal including sleep record

Case 3 T B a Caucasian newborn boy was the first child of a mother whose pregnancy had been complicated by urinary tract infection at 15 weeks and leaking membranes at 32 weeks Her labour started at term and was prolonged and delivery required outlet forceps Birth weight was 3300 g and Apgar scores were 2 at 1 min and 9 at 5 min after resuscitation The baby was well and alert during the first day but on the following day he refused to take feedings

On his third day of life the child developed apneic spells and twitchings and had tachycardia He was treated with kanamycin ampicillin and phenobarbital but his condition failed to improve On the fourth day he was transferred to the Vancouver General Hospital

On admission the child was comatose hyporeflexic and required assisted ventilation CSF samples were consistently sanguineous and showed markedly elevated protein level (7 95 g/l) The spinal fluid glucose concentration was 0 11 mmol/l contrasting with the blood glucose level of 8 mmol/l An EEG was nearly isoelectric and the CT scan showed blood in the dilated third and lateral ventricles He had a platelet count of only 9× 10^9/l He died 2 days after admission

At post mortem examination purulent partly hemorrhagic exudate filled the meninges over brain and spinal cord and *E coli* was grown from it The brain was severely swollen and partly autolyzed The cerebellar tonsils were necrotic and had become detached and displaced into the spinal canal

The lower thoracic and lumbosacral spinal cord was softened and hemorrhagic Microscopically pus and fragments of cerebellar tissue filled the subarachnoid space Polymorphonuclear leucocytes infiltrated the roots The lumbosacral spinal cord was entirely softened The nerve cells were still recognizable though necrotic In the lower thoracic region the softening was mainly in the grey matter with the white matter oedematous but otherwise intact The arteries were contracted In the sacral cord the anterior spinal artery was cut obliquely here the vessel was partly dilated and filled with recent thrombus which was just beginning to be organized Whilst pus surrounded the vessel the wall was not destroyed or markedly inflamed The smaller vessels within the necrotic areas of the spinal cord were distended with blood and many had leaked blood without actually being necrotic

DISCUSSION

Bacterial infections primarily involving the spinal cord are confined almost exclusively to 2 pathogens, *Mycobacterium tuberculosis* (2) and *Treponema pallidum* (8) The commonest infective cause of myelitis is viral infection and anterior horn cell damage due to poliomyelitis and occasionally Coxsackie virus is well recognized Though the common clinical picture in these viral infections is asymmetrical paralysis, symmetrical quadriplegia has also been described (17 21) Rubella infection (6) as well as rubella vaccination (12) have been reported as the cause of diffuse myelitis Rubella vaccination is not the only immunization procedure causing diffuse myelitis since it was also described after antirabies vaccination (20) Transverse myelitis or rather myelopathy (19) is well known in association with mycoplasma pneumoniae (15) Less frequently it may follow rubeola or varicella or even upper respiratory tract infection in which no organism has been identified (1) It can also follow diphtheria tetanus and poliomyelitis immunization (25) Parasites may invade the spinal cord and each year there are several reports of schistosomal myelopathies (18 22)

There are only 2 recent case reports of spinal cord involvement in bacterial meningitis

Acta Pædiatr Scand 69 475–479, 1980

GLYCOSYLATED HEMOGLOBIN (HbA_{1c}) AND PLASMA LIPOPROTEINS IN JUVENILE ONSET DIABETES MELLITUS

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ABSTRACT. Pollak, A., Widhalm, K., Havelec, L , Frisch, H. and Schober, E. (Division of Neonatology and Congenital Disorders, Department of Paediatrics and Institute for Medical Statistics and Documentation, University of Vienna, Vienna, Austria). Glycosylated hemoglobin (HbA_{1c}) and plasma lipoproteins in juvenile onset diabetes mellitus. Acta Paediatr Scand, 69 475, 1980 —Plasma lipid and lipoprotein levels and hemoglobin A_{1c} estimates of diabetic control were measured in 19 juvenile-onset diabetics (8 girls and 11 boys) upon admission (day 1) and at the end (day 25) of a 4-week summer camp, where the patients were put on a controlled diet with a da[illegible] linoleic acid intake of about 16 g Lipoproteins were also measured in 64 healthy controls [illegible] but significant decrea[illegible] M + S E.M , $p<0.05$) [illegible] cholesterol (1.0±0 07 [illegible] triglycerides (1.2+0 1 [illegible] found between hemog[illegible] period ($p<0.01$) How[illegible] lipids or lipoproteins [illegible] activity could have been responsible for the [illegible] tein cholesterol levels noted by the end of the camp.

KEY WORDS Juvenile-onset diabetes, glucose control, hemoglobin A_{1c}, lipoproteins

Raised blood lipids (1, 13) and/or decreased concentrations of high-density lipoprotein (HDL) (19, 22) may contribute towards the predisposition to coronary heart disease among the diabetic population (17, 18, 19) Diabetic control (6, 19), insulin therapy or diet (21) might influence HDL concentrations

In the present study plasma lipids (PL) and lipoproteins (PLP) were measured in a group of insulin-dependent juvenile diabetics (IDD) and compared with non-diabetic controls In the IDD group analyses were repeated after the children had spent four weeks at a summer camp and were put on a modified diet Hemoglobin A_{1c} (HbA_{1c}), which is considered to be an alternative indicator of diabetic control to plasma or urine glucose estimations, was also measured in the diabetic patients (5, 14) The aim of this study was to test whether any relationship exists between PL, PLP, in particular HDL cholesterol (HDL-C) and HbA_{1c} estimates of diabetic control

MATERIAL AND METHODS

Patients During a four week summer camp for 61 IDD, 19 patients (8 girls and 11 boys) aged 2 to 12 years, were randomly selected for further investigation Duration of diabetes was 1–10 years (mean 4 5) and the daily insulin dosage ranged from 0 2 to 1 4 IU/kg body weight (mean 0 8) All children took part in a daily physical activity program and were under close medical and dietary supervision 64 healthy children (31 girls and 33 boys) of a similar age range formed the control group The body weight in each case (IDD and controls) did not deviate by more than 10% from the normal value (23)

Study protocol HbA_{1c}, PL and PLP were determined upon admission (day 1) and at the end (day 25) of the summer camp The patients had all been on a modified diet at the camp which provided an average daily caloric supply of 11 732 kJ containing 35% of carbohydrates, 25% of proteins and 40% of fats 16 g of linoleic acid

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Submitted Nov 28 1979
Accepted Dec 6 1979

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ble 2 *Lipid and lipoprotein cholesterol concentrations in 19 juvenile diabetics upon admission to the camp and in 64 healthy controls*

	Total-C[a] (mmol/l)	HDL-C (mmol/l)	LDL-C (mmol/l)	VLDL-C (mmol/l)	TG[b] (mmol/l)
abetics					
Male (M ± S D)	3 5±0 8	1 1+0 4	2 2±0 7	0 2±0 2	1 2±0 6[d]
Range	1 7–4 4	0 5–1 8	0 8–3 1	0 10–0 5	0 6–2 1
n=11					
Female (M ± S D)	4 3±0 9[c]	0 9+0 2	3 0±0 8	0 3±0 3	1 2±0 5[d]
Range	2 6–5 3	0 7–1 5	1 9–4 4	0 1–0 8	0 6–1 9
n=8					
ontrols					
Male (M ± S D)	4 1±0 7	1 2+0 3	2 6±0 7	0 09±0 06	0 7±0 2
Range	2 5–5 6	0 6–1 9	1 8–4 1	0 01–0 3	0 4–1 5
n=33					
Female (M ± S D)	3 9±0 9	1 2+0 3	2 5±0 9	0 1±0 07	0 7±0 2
Range	2 2–6 9	0 5–1 8	0 7–5 5	0 02–0 4	0 4–1 3
n 31					

C cholesterol
TG=triglyceride
Significantly different from diabetic male ($p<0.05$)
[d] Significantly different from control ($p<0.001$)

RESULTS

Table 1 shows a slight but significant decrease in mean HbA₁c by the end of the study period There was no difference in HbA₁c between male and female diabetics either on day 1 or day 25 As shown in Fig 1 there was a significant linear correlation between urine glucose excretion during the study period and HbA₁c on day 25 No relationship was found between blood glucose and HbA₁c

Mean HDL C increased, while mean TG decreased significantly during the study period (Table 1) No correlation was found between HbA₁ PL and PLP nor between blood glucose PL and PLP either on day 1 or on day 25

Total TG were significantly higher in male and female diabetics upon admission to the camp as compared with non diabetic controls (Table 2) The values of mean total C and LP in diabetic males and females were similar to levels in the control group Mean total C was significantly higher in female than in male diabetics upon admission to the camp (Table 2) This difference was no longer apparent after four weeks on a modified diet at the camp

DISCUSSION

HbA₁c, which is elevated in diabetic patients (5, 14), reflects chronic hyperglycemia and, is, thus a valid index for assessing long term diabetic control (9, 14) Our findings of a significant linear correlation between HbA₁c and urine glucose excretion over a preceding period of four weeks is consistent with this concept

Since HbA₁c synthesis is thought to be a function of the mean blood glucose concentration over several weeks (14), one is tempted to relate the decrease in mean HbA₁c during the camping period to an overall improvement in diabetic control However, the individual data were quite heterogeneous Five children were even found to have increased HbA₁c levels This heterogeneity, as well as the relatively small magnitude of HbA₁c response may be related to the relatively short study period of four weeks

The mean total TG were found to be significantly higher in male and female diabetics on admission to camp than in controls This difference was no longer apparent at the end of the study period and may be due mainly to

Table 1 *Blood glucose (BG) hemoglobin A_{1c} (HbA_{1c}), lipid and lipoprotein cholesterol concentrations in 19 juvenile diabetics on day 1 of the study period compared with day 25*

I=day 1 of the study period II=day 25 of the study period

	BG[a] (mmol/l)	BG[b] (mmol/l)	HbA_{1c} (%)	Total C[c] (mmol/l)	HDL C (mmol/l)	LDL C (mmol/l)	VLDL C (mmol/l)	TG[d] (mmol/l)
I (n=19)								
Mean	11 3	13 6	10 4	3 8	1 0	2 5	0 2	1 2
S D	3 0	2 9	1 3	0 9	0 3	0 8	0 2	0 5
S E M	0 7	0 7	0 3	0 2	0 07	0 2	0 05	0 1
Range	7 9–16 8	6 8–18 3	8 0–12 7	1 7–5 3	0 4–1 8	0 8–4 4	0 005–0 8	0 6–2 1
II (n=19)								
Mean	10 7	12 2	9 8[e]	3 8	1 3[f]	2 4	0 2	0 7[g]
S D	3 8	4 3	1 7	1 2	0 4	0 9	0 3	0 2
S E M	0 9	1 0	0 4	0 3	0 1	0 2	0 07	0 04
Range	4 2–17 0	5 6–18 7	7 2–13 3	2 2–7 5	0 7–2 2	1 1–4 7	0 05–1 5	0 4–1 1

[a] Preprandial
[b] Postprandial
[c] C=cholesterol
[d] TG=triglyceride
[e] $p<0\ 05$
[f] $p<0\ 02$
[g] $p<0\ 001$
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were given daily which caused a polyunsaturated/saturated (P/S) ratio of 0 37 After an overnight fast of at least 10 hours venous blood samples were taken between 7 00 and 8 00 a m before injecting the morning insulin dose

24 hour urine glucose excretion was determined daily capillary blood glucose (fasting and postprandial) six times each on day 1 and day 25 In the control group venous blood samples were taken after an overnight fast Informed written consent was obtained from all parents

Methods Specimens were collected in heparin tubes and immediately transported on ice for analysis at the department (transport time about 1 5 hours) Cholesterol (C) and triglycerides (TG) were determined on the same day by means of full enzymatic methods (4 24) Quality control with the lipid standard serum Precilip[R] revealed a coefficient of variation of 2 to 3% Lipoprotein fractionation was performed using a preparative ultracentrifuge according to the LCR method NIH (20)

HbA_{1c} was measured by the high performance liquid chromatography method on undialysed hemoglobin solution (8) All samples were run in duplicates The coefficient of variation ranged from 4 5 to 7 5% for high and low levels respectively Results were expressed as % of total hemoglobin Blood glucose was measured by the glucose oxidase method and urine glucose polarimetrically

Statistical methods p Values were obtained by means of the Student s t test and simple linear regression analysis

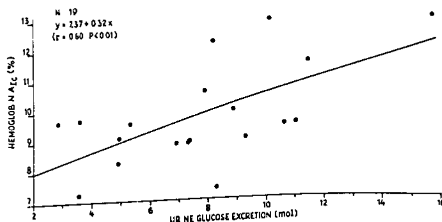


Fig 1 Correlation between urine glucose excretion (cumulative values during the four week summer camping) and HbA_{1c} measured on day 25 of the summer camping

Table 2 *Lipid and lipoprotein cholesterol concentrations in 19 juvenile diabetics upon admission to the camp and in 64 healthy controls*

	Total C[a] (mmol/l)	HDL-C (mmol/l)	LDL-C (mmol/l)	VLDL-C (mmol/l)	TG[b] (mmol/l)
Diabetics					
Male (M ± S.D.)	3.5±0.8	1.1±0.4	2.2±0.7	0.2±0.2	1.2±0.6[d]
Range	1.7–4.4	0.5–1.8	0.8–3.1	0.10–0.5	0.6–2.1
n=11					
Female (M ± S.D.)	4.3±0.9[c]	0.9±0.2	3.0+0.8	0.3±0.3	1.2±0.5[d]
Range	2.6–5.3	0.7–1.5	1.9–4.4	0.1–0.8	0.6–1.9
n=8					
Controls					
Male (M ± S.D.)	4.1±0.7	1.2±0.3	2.6±0.7	0.09±0.06	0.7±0.2
Range	2.5–5.6	0.6–1.9	1.8–4.1	0.01–0.3	0.4–1.5
n=33					
Female (M ± S.D.)	3.9±0.9	1.2±0.3	2.5+0.9	0.1±0.07	0.7±0.2
Range	2.2–6.9	0.5–1.8	0.7–5.5	0.02–0.4	0.4–1.3
n=31					

RESULTS

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Mean HDL-C increased, while mean TG decreased significantly during the study period (Table 1). No correlation was found between HbA$_{1c}$, PL and PLP, nor between blood glucose, PL and PLP, either on day 1 or on day 25.

Total TG were significantly higher in male and female diabetics upon admission to the camp as compared with non-diabetic controls (Table 2). The values of mean total C and LP in diabetic males and females were similar to levels in the control group. Mean total C was significantly higher in female than in male diabetics upon admission to the camp (Table 2). This difference was no longer apparent after four weeks on a modified diet at the camp.

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Since HbA$_{1c}$ synthesis is thought to be a function of the mean blood glucose concentration over several weeks (14), one is tempted to relate the decrease in mean HbA$_{1c}$ during the camping period to an overall improvement in diabetic control. However, the individual data were quite heterogeneous. Five children were even found to have increased HbA$_{1c}$ levels. This heterogeneity, as well as the relatively small magnitude of HbA$_{1c}$ response may be related to the relatively short study period of four weeks.

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Range	7 9–16 8	6 8–18 3	8 0–12 7	1 7–5 3	0 4–1 8	0 8–4 4	0 005–0 8	0 6–[illegible] 1
II (n=19)								
Mean	10 7	12 2	9 8[e]	3 8	1 3[f]	2 4	0 2	0 [illegible]
S D	3 8	4 3	1 7	1 2	0 4	0 9	0 3	0 2
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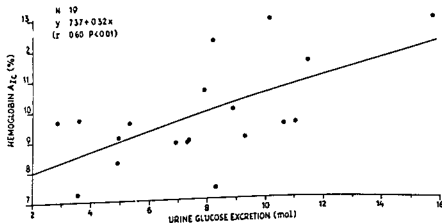


Fig. 1 Correlation between urine glucose excretion (cumulative values during the four week summer camping) and HbA_{1c} measured on day 25 of the summer camping

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Submitted July 30 1979
Accepted Dec 15 1979

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nutritional factors, particularly to the low intake of saturated fatty acids (16%) (25) and carbohydrates, moreover, the diet contained a high proportion of starch and non-absorbable fiber in the form of vegetables and fruit, factors known to lower serum TG (2) The levels of total C in female and male diabetics were similar to the values in the control population Kennedy et al (18) observed higher total C levels in male diabetics than in a non diabetic group of patients with a mean age of over 40 No relationship was found between HbA_{1c} estimates of diabetic control and either TG or total C, in contrast to the findings of Ditzel et al (10) in ten newly-diagnosed diabetics

Data on lipoproteins in juvenile onset diabetics are scanty Chase et al (7) found increased LDL and decreased HDL levels This pattern is generally accepted to be associated with an increased risk of premature athero sclerosis (17, 22) In the present study a trend was observed towards diabetics having lower HDL levels than controls This observation was not, however, statistically significant when analyses were carried out separately for boys and girls or both sexes combined, as reported previously (11) Hence subsequent data on HDL levels were combined for boys and girls

By the end of the study period HDL C had increased significantly in the diabetic patients to levels similar to those in the control group It has been claimed that low HDL concentrations are related to poor diabetic control (6, 19) Calvert et al (6) found an inverse correlation between HbA_{1c} and HDL in a mixed diabetic population However in the present study the paired data analysis did not show an improvement in HbA_{1c} estimates of diabetic control concomitant with an increase in HDL-C in accordance with others (3, 12 18) It must be mentioned, however that no extreme HbA_{1c} levels were measured since no patients in metabolic decompensation were included Although the relatively short duration of this study must be emphasized other factors independent of glucose control have to be considered, thus the daily physical activity program could indeed be responsible for the rise of HDL levels (15)

HbA_{1c} estimates of diabetic control are probably not related to PL or PLP in juvenile onset diabetes Data from longlasting studies are necessary to define clearly the effect of dietary regimen and physical activity on lipid and lipoprotein metabolism

ACKNOWLEDGEMENTS

This study was supported by Forschungsauftrag des Bundesministeriums fur Gesundheit und Umweltschutz Zl II-52 173/3-66/78 and by Jubilaumsfondsprojekt Nr 1406 der Österr Nationalbank

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Acta Paediatr Scand 69 481–483, 1980

HAEMOGLOBIN A_{1c} A PREDICTOR FOR THE DURATION OF THE REMISSION PHASE IN JUVENILE INSULIN DEPENDENT DIABETIC PATIENTS

U VETTER E HEINZE W BEISCHER E KOHNE E KLEIHAUER and W M TELLER

From the Centers of Paediatrics and Internal Medicine University of Ulm (Donau) F R G

ABSTRACT Vetter, *U*, Heinze, *E*, Beischer, *W*, Kohne, E., Kleihauer, E and Teller, W M (Centers of Paediatrics and Internal Medicine, University of Ulm, FRG) Haemoglobin A_{1c} a predictor for the duration of the remission phase in juvenile insulin dependent diabetic patients Acta Paediatr Scand, 69 481, 1980 —Increased HbA_{1c} concentrations in diabetic patients indicate retrospectively a poor metabolic control during the preceding 2–3 months In the present study attempts have been made to use the HbA_{1c} concentration at the time of diagnosis as an indicator of the duration of the remission phase in 23 juvenile diabetic children *The regression analysis revealed a significant negative correlation between* the initial HbA_{1c} concentrations and the duration of the remission phase defined as no glucose excretion, an insulin requirement of less than 0 5 U/kg/day and detectable serum C-peptide concentration ($r=-0\ 84$, $p<0\ 001$) The results suggest that the initial HbA_{1c} concentration may serve as a useful indicator to predict the duration of the remission phase in juvenile onset diabetic patients

KEY WORDS Haemoglobin A_{1c}, remission phase, juvenile-onset diabetes

Haemoglobin A_{1c} (HbA_{1c}) is a glycosylated fast moving minor haemoglobin component In diabetic patients the HbA_{1c} concentration *indicated the quality of the metabolic control* during the preceding 2–3 months (3, 4, 5, 8, 9 12) Furthermore it has been well established that strict treatment of the diabetic patients for 2–3 months can normalize the HbA_{1c} concentration (2 12) In the present *study the HbA_{1c} concentration in 23 newly* diagnosed diabetic children was used to predict the duration of the *remission phase*

PATIENTS AND METHODS

Patients

All patients with newly discovered diabetes mellitus from the Center of Paediatrics University of Ulm F R G who were diagnosed and treated between 1 2 1977 and 15 12 1978 were studied prospectively The pertinent clinical *and laboratory data of the 23 children are pre*sented in Table 1 Treatment was initiated with an appropriate diet and two daily injections of an individual mixture of regular and an intermediate insulin (N P H) All patients went into remission defined as the time after diagnosis with an insulin requirement of less than 0 5 U/kg/day a detectable serum C peptide concentration and no urinary glucose excretion (7 10) After discharge *from the hospital all patients tested their urinary glucose* excretion three times per day with Clinitest*

Methods

Blood glucose was measured with a hexokinase method and urine glucose excretion was determined polarimetrically (13) Serum C-peptide was determined by a radioimmunoassay (1) Haemoglobin A_{1c} was measured by column chromatography (6) Every 2–6 weeks the patients collected a 24-hour urine sample at home utilizing random sampling i e in the *morning afternoon and at night*

RESULTS

In Table I are shown sex, age of onset, the initial but not the fasting blood glucose, the duration of the remission phase, the C peptide concentration and the insulin *requirement* during the remission phase and the initial HbA_{1c} concentration for the 23 newly diagnosed *diabetic children*

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RESULTS

In Table 1 are shown: sex, age of onset, the initial but not the fasting blood glucose, the duration of the remission phase, the C-peptide concentration and the insulin requirement during the remission phase and the initial HbA_{1c} concentration for the 23 newly diagnosed diabetic children

Table 1 *Clinical data of 23 patients with newly discovered diabetes mellitus*

Case	Sex	Age at onset (years)	Initial blood glucose (mmol/l)	C peptide during remission (ng/ml)	Insulin requirement during remission (μ/kg/day)	HbA_{1c} (%)	Duration of remission (months)
1	F	12	33 3	0 9	0 3	11 7	4
2	F	11	23 9	2 2	0 4	12 7	4
3	F	14	25 0	1 6	0 4	4 8	15
4	F	13	22 2	2 2	0 4	14 4	3
5	F	7	14 4	2 0	0 2	3 6	13
6	F	10	19 4	2 3	0 3	9 1	7
7	F	3	23 0	1 8	0 2	4 9	12
8	F	8	10 0	1 7	0 4	6 9	6
9	F	13	15 0	0 8	0 3	8 8	5
10	F	6	17 8	1 9	0 3	8 2	7
11	F	9	13 9	1 9	0 2	5 9	9
12	F	2	22 2	2 0	0 2	8 3	9
13	F	12	23 3	0 9	0 4	10 3	4
14	M	8	41 1	1 3	0 4	4 6	12
15	M	14	16 7	2 6	0 4	9 0	4
16	M	10	14 4	2 8	0 3	8 4	9
17	M	11	22 2	3 2	0 3	4 2	12
18	M	13	20 5	1 3	0 4	14 2	5
19	M	14	33 3	1 0	0 3	8 9	7
20	M	15	12 2	1 2	0 2	9 2	3
21	M	6	19 2	1 0	0 4	8 4	7
22	M	7	50 5	2 5	0 4	9 1	9
23	M	3	17 2	1 5	0 3	8 5	8

The duration of the remission phase was not dependent on sex, age or the blood glucose concentration measured on admission to the hospital However, the regression analysis revealed a significant negative correlation between the initial HbA_{1c} concentration and the duration of the remission phase ($r = -0\ 84$, $p < 0\ 001$) Fig 1 shows that high initial HbA_{1c} concentrations (range 9–13 %) were followed by a short remission of 3–5 months while comparatively low HbA_{1c} concentrations (range 4–6 %) were followed by a remission phase of 10–15 months The partial regression analysis did not detect any influence of sex or age on the correlation between HbA_{1c} and the duration of the remission phase

DISCUSSION

In the last few years diabetes mellitus in children has been diagnosed earlier during the natural course of the disease with the result that about 60 % of patients reach a remission phase, while during 1960–70 a partial or complete remission was observed in less than 10 % of insulin dependent diabetics (10) The report seems to indicate that the severity of the metabolic disturbance at the onset of juvenile insulin dependent diabetes mellitus determines at least in part the remission phase which has been shown previously by clinical observations (7, 11) On the other hand it is well accepted that in juvenile and adult patients with longstanding diabetes mellitus the HbA_{1c} concentration indicates the metabolic control during the previous months (3 4, 5, 8 9, 12)

In newly diagnosed insulin dependent diabetics the fasting blood glucose correlated with the initial HbA_{1c} concentration (2) In the present study no significant correlation existed between the initial HbA_{1c} concentration and the blood glucose on admission to hospital This confirms a previous report, where, in addition, no correlation was found between

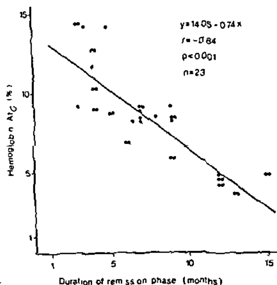


Fig 1 Correlation between initial HbA_{1c} concentration (%) and duration of remission phase (months)

the initial HbA_{1c} concentration and the pH or the base excess (5) However, our results revealed a significant correlation between the initial HbA_{1c} concentration in 23 newly diagnosed diabetic children and the duration of the remission phase We therefore suggest that the HbA_{1c} concentration at the onset of juvenile diabetes mellitus may be used as a simple indicator to predict the individual duration of the remission phase and may serve as a valuable tool in the rational control of ambulatory diabetic patients

ACKNOWLEDGEMENTS

We thank Professor R D G Milner Children s Hospital, University of Sheffield, England who corrected the language of the manuscript Furthermore, the expert technical assistance of Miss M Krause and Mrs U Weinstein and the secretarial help of Mrs J Oberem is gratefully acknowledged

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Submitted Aug 13, 1979
Accepted Jan 18, 1980

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Acta Paediatr Scand 69 485–489, 1980

PARIETAL CELL ANTIBODIES AND GASTRIC SECRETION IN CHILDREN WITH DIABETES MELLITUS

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ABSTRACT Kokkonen, J (Department of Paediatrics, University of Oulu, Oulu, Finland) P[illegible] ch ldren with diabetes mellitus Acta Paediatr S[illegible] in group A as compared with the control group Two patients [illegible] 17 patients out of the 41 studied had hyposecretion and one achlorhydria The result became most obvious in the group with a duration of diabetes over 10 years, where MAO was significantly diminished ($p<0.05$) Gastric morphology revealed atrophic gastritis in 3 patients from seven biopsies in group A and one out of five biopsies for severe hyposecretion in group B Two other children in group A had superficial gastritis Serum ferritin levels decreased along with the duration of diabetes Those with gastritic mucosa had the lowest values

KEY WORDS Diabetes mellitus ferritin gastric juice, gastric mucosa, gastrin, parietal cell antibody

According to a number of investigations circulating parietal cell antibodies (PCA), gastric hyposecretion and chronic gastritis occur more frequently in patients with diabetes mellitus than in normal subjects (1, 7, 8, 9, 17) In a diabetic patient there are many factors which may reduce gastric acid secretion hypo and hyperglycemia (11 12), transiently high glucagon concentrations (4) and increased susceptibility for chronic gastritis (1) Although well documented in adults, the changes in the gastric mucosa of diabetic children are less well known

The aim of the present study was to investigate the frequency of circulating parietal cell antibodies and gastric hyposecretion with special attention to the relationship between these findings and the mucosal morphology in diabetic children

MATERIAL AND METHODS

Subjects for screening PCA

147 children with juvenile onset diabetes mellitus attending regular follow up at the Department of Paediatrics University of Oulu were screened for circulating PCA 71 of them were females and 76 males (mean age 12 years range 4–18 years and mean duration of illness 5 years range 0–15 years)

Subjects for gastric stud es

Group A comprised 8 diabetics with circulating PCA in their serum Their ages duration of illness and sex are given in Table 1 Two of these had a concomitant autoimmune disorder (thyroiditis vitiligo)

Group B consisted of 41 PCA negative diabetics selected from 139 screened 15 of them were in patients hospitalized for the treatment of their disease between October 1976 and the end of December 1977 and the other 26 were randomly selected and investigated during regular visits to the outpatient department Their ages duration of illness and sex are given in Table 1 Five of them had an associated disease (3 with coeliac diseases one with thyroiditis and one with asthma) This group was divided into three subgroups according to the dura

Table 1 *Age duration of diabetes, sex, serum ferritin and vitamin B_{12} in diabetic children studied for gastric secretory function*
Group A Diabetics with parietal cell antibodies (PCA) group B without PCA Means and ranges are given f = female m = male

Group	PCA	No	Sex	Age (years)	Duration of of diabetes (years)	S Ferritin (µg/l)	S Vitamin B_{12} (pmol/l)
A	+	8	4f 4m	14 04 7–17	7 25 0–13	57 7–130	718 440–1000
B	−	41	24f 14m	14 60 7–18	7 90 0–15	69 4–260	855 480–1940

tion of diabetes B_1 1–4 years B_2 5–9 years and B_3 over 10 years The clinical data with the laboratory findings in groups A and B are given in Table 1

A control group C for gastric secretion and the level of fasting serum gastrin was composed of 12 non diabetic children (5 girls 7 boys aged 6–16 years mean 12 47 years) Three of these children underwent a duodenojejunal biopsy following suspicion of malabsorption with normal findings The others were treated in the hospital for functional abdominal pains without signs of malabsorption

Methods

The clinical balance was evaluated by measuring the percentage excretion of carbohydrates in the urine from the recommended diet This was done by collecting the 24 hour urine at home on three separate occasions The daily insulin demand was calculated in units per kilogram of body weight at the time of the examination

Laboratory tests Circulating PCA were studied at a serum dilution of 1 10 by utilising antihuman immunoglobulin fluorescein conjugate (Roboz®) RIA methods were used to study the serum gastrin (CIS kit®) and ferritin (at the laboratory of the Paediatric Research Foundation Helsinki) and vitamin B_{12} (Phadebas®) levels Normal values for ferritin were taken to be 7–142 µg/l (14) and for B_{12} 200–600 pmol/l (10)

Gastric function The output of gastric juice was studied by the standard method of pentagastrin stimulation (Peptavlon® ICI 6 µg/km body weight) The studies were performed after an overnight fast and in the absence of signs of acidosis or acute symptoms The collection tube was

after the administration of pentagastrin to determine maximal acid output (MAO) The volume of gastric juice and the basal and maximal acid output were measured Measurements of acid output were expressed in µmol/h/kg body weight Values below 1 S D of the control group were considered hypoclorhydric and below 10 µmol/h/kg achlorhydric

Gastric morphology Gastric biopsy samples were obtained from patients with circulating PCA (group A) and from the patients without PCA but with over 10 years duration of diabetes and with gastric hyposecretion A Wolff multipurpose capsule or a paediatric Crosby Kugler capsule was used The biopsies from the corpus were taken under fluoroscopic control Routine histological

the inflammatory changes were in the superficial epithelium the gastric pit region and the related lamina

Table 2 *Gastric acid secretion and fasting serum gastrin concentrations in controls and in children with diabetes mellitus*
A Diabetics with circulating PCA B without PCA Means ±S D

	Controls $n = 12$	A $n - 8$	B $n - 41$
Basal secretion volume (ml/h)	59±42	57±59	56±34
HCl output BAO (µmol/h/kg)	71+42	32*±48	47+41
Maximum secretion volume (ml/h)	156±43	137±121	147+75
HCl output MAO (µmol/h/kg)	424±119	248*±199	353+207
Gastrin (pmol/l)	32±7	103**±113	40+?2

* $p<0$ 05 compared with the controls
** $p<0$ 01 compared with the controls

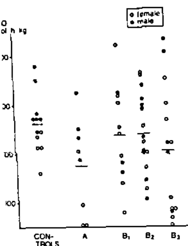


Fig 1 Maximum acid output (MAO) in controls and diabetic children with PCA (*A*) and without PCA (*B*) in year duration groups B_1 0–4 years B_2 5–9 years and B_3 over 10 years of duration Mean (—) ± 1 S D (---) are given

atrophia and atrophic when the changes affected the glandular layer The severity of atrophy was classified as mild moderate or severe according to the loss of the tubules

Statistical methods A Mann Whitney nonparametric test was used in the statistical analysis of the data

RESULTS

Parietal cell antibodies

Circulating PCA were found in 8 (4 girls and 4 boys) of 147 patients (5 4%) Neither the mean age (14 04 years) nor the duration of the diabetes (7 25 years) was significantly different from the whole group screened (12 48 and 5 33 years respectively) The girls with gastritis had particularly high titres only one boy had gastritis and his circulating PCA concentration was not noticeably high

Gastric acid secretion

Wide variations can be seen in both BAO and MAO in all the groups (Table 2) Although both the BAO and MAO were clearly diminished in both the study groups the mean volumes remained nearly normal The girls of the control group had a lower MAO than the boys (Fig 1)

In group A, with circulating PCA, both the BAO and MAO were significantly ($p<0\ 05$) decreased as compared with the controls Two girls had achlorhydria, whereas hypochlorhydria was recorded in one girl and one boy The remaining 4 patients had normal secretion (Fig 1)

In group B the decrease in the BAO and MAO was not statistically significant In the whole group however, 17 patients (11 girls and 6 boys) out of the 41 studied (41%) had a lowered MAO as compared with the controls A distinct group of hyposecretors can be identified early in the course of the diabetes In a group with diabetes duration over 10 years (B_3) 6 out of 14 patients (43%) had severe hypochlorhydria, and the rest a normal or even increased MAO (Fig 1)

Fasting serum gastrin

The highest values of serum gastrin were recorded in patients with circulating PCA and hypochlorhydria In group A the gastrin level was also significantly raised ($p<0\ 01$) Only a few patients with hypochlorhydria in group B had elevated fasting serum gastrin values

Serum ferritin and vitamin B_{12}

The mean values of serum ferritin in the two study groups were higher (Table 1) than the normal values (14) Group B showed a decrease in ferritin with time (Table 3) Those with atrophic gastritis had the lowest values

The patients with gastric hyposecretion in group B exhibited no significant decrease of

Table 3 *Serum ferritin concentrations in different diabetesduration groups of children without PCA*

B 1–4 years B_2 5–9 years and B_3 over 10 years of duration Means ± 1 S D are given

	No	Duration of diabetes (years)	S Ferritin (µg/l)
B_1	11	3 13±1 34	103±54
B_2	15	7 60±1 47	66±69
B_3	14	12 02±1 54	46*±45

* $p<0\ 05$ as compared with group B (*t* test)

Table 4 *Gastric biopsy results in diabetic children with PCA (A) and without PCA but severe hypochlorhydria (B)*

	N	Normal	Superficial gastritis	Atrophic gastritis
A	7	2	2	3
B	5	4	0	1

serum ferritin as compared with the whole group No deficiency of vitamin B_{12} was found

Clinical balance

Those diabetics with PCA or severe hypochlorhydria had no significant increase in their daily carbohydrate excretion or insulin demand

Gastric morphology

Altogether, 12 diagnostic samples, one from each patient, were obtained In group A with circulating PCA, 3 patients had mild or moderate atrophic gastritis (Table 4) Another 2 patients had severe gastritis, but the samples were too superficial to allow classification of the degree of atrophy Only one patient, with decreased MAO but without PCA, out of 5 biopsied had moderate atrophic gastritis The rest were normal

DISCUSSION

Circulating PCA occur in type A gastritis (15) Some authors consider these antibodies to be merely a humoral response to a destroyed parietal cell, whereas others have found them to actively reduce gastric secretion (2 5) The incidence, of below 3% under 20 years of age has been reported mostly in unselected subjects (3, 6, 7) In another study (to be published), only 2 PCA positive subjects were found among 164 school-aged children The value of 5 4% in this series suggests an increased incidence of PCA in diabetic children Their presence seems to be independent of sex although the girls exhibit more severe gastric changes Contrary to the findings in adults (2) 2 boys with marked PCA titre revealed normal corpus mucosa

Severe gastric hyposecretion was found in 43% of longstanding diabetic children (over 10 years) Since there were more females than males in that subgroup, the true figure is actually lower, as the females more often had hypochlorhydria Angerwall (1) has reported hypochlorhydria in 30% of adult diabetics It therefore seems that two-thirds of diabetics have an intact gastric secretory function throughout their lives and one-third develop hypochlorhydria This seems to become manifest 10 years after the onset of diabetes

Gastric hyposecretion is usually attributed to the underlying gastritis (18) In the single published study on the incidence of chronic gastritis in children and young adults (13) a quarter of 2–20-year-olds showed gastritis mostly mild forms In this series corpus gastritis was found in 5 children with circulating PCA and in one without Hypochlorhydria without PCA, may be related to type B gastritis (15) Unfortunately the biopsies were taken without using gastroscopy to visualize the antral mucosa

Hypoglycemia (11), hyperglycemia (12) and iron deficiency (2) also inhibit gastric acid secretion but the long term effect of the first two is not known Reduced concentrations of serum ferritin were recorded in only a few patients with atrophic gastritis and may be considered to be an indicator of increased iron loss (16) As, in general a good iron balance was observed in the diabetics of this series iron deficiency cannot be considered a serious factor in hypoacidity High glucagon concentrations may also predispose the patient to type B gastritis by relaxing the pyloric sphincter

ACKNOWLEDGEMENT

I am grateful to Dr R Herva who helped to evaluate the biopsy samples and Miss A Pikkarainen who collected the gastric samples

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Submitted Oct 4 1979
Accepted Jan 15 1980

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Acta Pædiatr Scand 69 491–498, 1980

ENDOGENOUS CLEARANCE RATE AND SYNTHESIS OF ALPHAFOETOPROTEIN DURING AND AFTER BLOOD EXCHANGE TRANSFUSION IN HYPERBILIRUBINAEMIC NEWBORNS

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ABSTRACT Teppo, A M and Simell, O (Department of Paediatrics, University of Helsinki Helsinki, Finland) Endogenous clearance rate and synthesis of alphafoetoprotein during and after blood exchange transfusion in hyperbilirubinaemic newborns Acta Paediatr Scand, 69 491, 1980.—Plasma alphafoetoprotein (AFP) concentration was monitored in 22 hyperbilirubinaemic infants during blood exchange transfusion at the age of 0–4 days, in order to determine the endogenous clearance rate and demonstrate possible postnatal synthesis of AFP The rapid fall in plasma AFP during the transfusion to 19±4% (mean ±1 S D) was followed by an increase to 81±15% of the initial concentration, only a 68% rise was expected after the establishment of a new equilibrium between the intra and extravascular pools of AFP Repeated transfusions in the same individuals gave similar findings In two infants the total amount of AFP in these pools increased by 4 0–7 4 mg after the transfusions The results could be accounted for by postnatal AFP synthesis Because of the diffusion of AFP from the extravascular compartment to the intravascular pool the actual turnover rate of AFP could not be measured The estimated magnitude of endogenous turnover rate of AFP, 0 02–0 09 min ¹, gave a mean half life of about 20 min, which is distinctly shorter than that of AFP in plasma (3–5 days) this could be due to either continuous synthesis of AFP or transfer of presynthesized AFP from the liver cells to extracellular pool We postulate that either the synthesis or the turnover rate of AFP is controlled by the plasma AFP concentration via a negative feedback mechanism

KEY WORDS Alphafoetoprotein, neonatal hyperbilirubinaemia, blood exchange transfusion endogenous clearance rate of AFP

Alphafoetoprotein (AFP) is synthesized during the proliferation of hepatocytes in the foetal liver (12 21) The concentration of AFP in foetal plasma is highest during the 13th gestational week and thereafter decreases to one tenth (mean 100 mg/l) towards delivery The concentration varies greatly at birth, as does its subsequent disappearance rate from the plasma About 80% disappears during the first 4–5 hours and has a $T_{\frac{1}{2}}$ of about 2 hours (9) During the following days the concentration remains almost unchanged and then decreases to the adult level in about a month, with a mean $T_{\frac{1}{2}}$ of approximately 5 days (9, 15)

The physiological function of AFP in the foetus is unknown It is believed to influence growth (9, 16) or to be a binder of either bilirubin (27) or oestrogens (7, 29) Rat and mouse but not human AFP binds oestrogen strongly and specifically (18, 25, 29) Some workers have found that human AFP is immunosuppressive (6, 13, 14), although not all agree on this point (20) Postnatal synthesis of AFP has been demonstrated in piglets, rats, and mice (8, 20), and indirect evidence suggests AFP synthesis after birth in man also (9 10, 17, 22) The serum of adult mice and pigs contains a thermolabile factor which, if injected into newborn animals inhibits AFP synthesis (2, 24) No such factor has been reported in adult human blood

Blood transfusions offer an opportunity to study these problems In this study we determined the magnitude of endogenous turnover

Table 1. *Pertinent data on the subjects of the study and the changes in plasma AFP during and after transfusions*

Case	Sex	Birth-weight (g)	Gesta-tional age (weeks)	Age at the first exchange (days)	Reason for exchange transfusion	Concentration of AFP (mg/l) Before trans-fusion	After trans-fusion	In new equi-librium	In new equi librium % of initial
Group I									
1	M	3 480	39	3	Rh immunization	102	13		
2	F	3 710	41	0	ABO immunization	36	7		
3	M	4 170	37	2	Rh immunization	116	15		
4	M	2 750	39	1	Rh immunization	290	55		
5	M	3 530	39	3	Hyperbilirubinaemia	31	8		
6	M	2 680	40	1	Rh immunization	120	36		
7	M	2 720	35	1	ABO immunization	200	40		
8	M	2 900	40	2	Hyperbilirubinaemia	68	15		
9	M	2 850	36	3	Hyperbilirubinaemia	202	26		
10	F	2 400	38	3	Hyperbilirubinaemia	59	15		
11	F	2 890	36	3	ABO immunization	204	36		
12	M	2 680	37	4	Hyperbilirubinaemia	69	13		
Mean ± S D		3 060±530	37±2	2 2		126±80	23±15		
Group II									
1	M	1 320	31	2	Hyperbilirubinaemia	924	90	690	75
2	F	1 040	29	4	Hyperbilirubinaemia	325	90	240	74
3	M	3 500	38	0	Rh immunization	160	30	175	109
4	M	2 680	37	4	Hyperbilirubinaemia	69	13	50	73
5	M	4 880	38	3	Hyperbilirubinaemia	96	20	56	58
6	M	2 930	35	0	ABO immunization	70	16	47	67
7	M	4 010	38	0	ABO immunization	51	10	42	81
8	F	3 530	40	0	ABO immunization	43	7	41	95
9	M	3 600	38	2	ABO immunization	55	10	47	85
10	M	1 550	33	0	Rh immunization	180	27	160	88
Mean ± S D		2 800±1 230	36±4	1 7		197±269	32±31	154±201	81±15

rate of AFP in hyperbilirubinaemic infants who had blood exchange transfusion(s) We found convincing evidence that AFP was synthesized after the blood exchanges

PATIENTS AND METHODS

Patients

The total series (Table 1) comprised 21 infants with gestational ages of 29–41 weeks who underwent blood exchange transfusions at the age of 0–4 days because of hyperbilirubinaemia due either to liver insufficiency (9 infants, 2 girls) or to isoimmunization (12 infants 3 girls) The plasma level of AFP during transfusions was monitored in 12 newborns (group I, 3 girls) The plasma concentrations of AFP and albumin were measured after exchange transfusions in another group of 10 infants (group II, 2 girls) One of the newborns was included in both groups

The exchange transfusions lasted 40–165 min, and the volume exchanged was 2 5–2 9 times the estimated blood volume of the infants (mean 620 ml of blood, range 280–840 ml) In group I the blood removed was collected in samples of 40 ml The times and volumes exchanged were carefully recorded Samples of donor blood were also taken and AFP and haematocrit analysed in all specimens

In group II the exchanges were performed as described for group I Blood samples for AFP and albumin determinations were taken at the beginning and end of the transfusions In addition 10–20 samples were taken during the following 8 days One infant had two transfusions, one had six and one had nine

Protein determinations

AFP concentration in the plasma of the infants was determined by electrophoresis in agarose gel containing antibodies as described by Laurell (11) Antiserum against AFP and standard serum were obtained from Behringwerke AG The detection limit was 0 5 mg/l with the

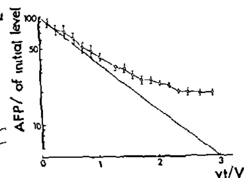


Fig. 1. Serum AFP concentrations during blood exchange transfusion as percentages of the initial level (mean + S.D.) in 12 newborns (Group I, Table 1) as a function of exchanged blood volume (expressed as vt/V, where v=exchange rate in ml/min, t=time in min, V=blood volume of the newborn in ml). The straight line gives the theoretical concentration assuming that AFP does not shift from the extravascular compartment to the blood during the transfusion.

0.5% antiserum used. The AFP in the plasma of the donors was measured by radioimmunoassay (17) using the same standard serum as above. Albumin was measured according to the bromcresol green binding method of Doumas (3).

Calculations

The theoretical dilution curve of the AFP concentration in plasma during blood exchange transfusions was calculated according to the formula

$$p = p_0 e^{-at} \qquad (1)$$

where p = AFP concentration at time t, p_0=pretransfusional AFP concentration in the infant's plasma, t= time in min from the commencement of transfusion, a=exchange rate coefficient (v/V), where v = amount of plasma exchanged in 1 min and V=plasma volume of the infant (8% of body weight×(1 Hct)). The calculation was based on the assumption that no intermixing of AFP occurs between the extravascular and intravascular proteins pools and AFP is not catabolized during the exchange.

The endogenous removal rate of AFP (λ) during the exchange transfusions was calculated according to Siimes et al. (23) in the Helsinki University Computer Centre from the equation

$$p = \frac{\lambda p_0 + a p_0 e^{-(\lambda+a)t}}{\lambda + a} \qquad (2)$$

using a non linear least squares fitting procedure. The AFP concentration in the donor plasma (<0.020 mg/l) was regarded as negligible as compared with that of the infants (mean 126 mg/l). The mean difference in haematocrit between neonates and donors was 3%; this difference was not taken into account in the calculations. Finally, the equation used for calculation of the half life of plasma AFP was

$$T_{\frac{1}{2}} = \ln 2/\lambda \qquad (3)$$

RESULTS

Changes during transfusions

The plasma AFP concentration decreased during the blood exchange transfusions in Group I at first parallel to the theoretical dilution curve, but gradually it began to deviate more from the expected value and finally almost formed a plateau (Fig. 1). At the end of the exchange the concentration of AFP was 19±4% of the initial value. Infants with a gestational age of ≤36 weeks had a somewhat higher final level (21±6%, n=3) than those with a gestational age of >36 weeks (16±3%, n=9). The final AFP level correlated positively (r=0.540, p<0.05) with the duration of the transfusion and was unrelated to the volume exchanged. This was evidently due to diffusion of AFP from the extravascular to the intravascular pool during slow transfusions.

The mean endogenous AFP removal rate (λ) was 0.043 min^{-1} (range 0.02–0.09 min^{-1}) giving a half life for AFP of 20 min (8–35 min) (Table 2). No correlation was found between the half lives and the gestational ages or birth weights. The λ values of infants with different aetiologies of hyperbilirubinaemia did not differ.

Steady state conditions were assumed to exist during the period of transfusion in the calculation of λ. This means that the amount of AFP released into the circulation from the extravascular pool should be equal to the amount catabolized, i.e. diffusion of AFP into the intravascular pool during transfusion should be negligible in spite of the concentration difference. However, owing to either the low molecular weight of AFP or the great difference in its concentration between the pools, or both, this was not the case (Fig. 1). Hence, the values for the endogenous removal rates of AFP are probably too high by a factor of 2–3, and the values calculated for $T_{\frac{1}{2}}$ too low.

Table 2. The endogenous removal rate (k) and half-life of AFP in twelve newborns

The values for k and $T_{\frac{1}{2}}$ were calculated assuming that AFP was neither catabolized nor shifted from the extravascular to the intravascular pools during the transfusion. For details, see results and discussion

Case	Duration of exchange (min)	Vol exchanged/ blood vol	k (min⁻¹)	$T_{\frac{1}{2}}$ (min)
1	55	2.7	0.037	19
2	110	2.5	0.020	35
3	50	2.6	0.032	22
4	115	2.5	0.047	15
5	165	2.7	0.020	35
6	105	2.5	0.031	22
7	75	2.6	0.030	23
8	55	2.8	0.080	9
9	65	2.8	0.029	24
10	95	2.9	0.052	13
11	40	2.8	0.047	15
12	40	2.7	0.090	8
Mean ± S.D.	81±38	2.7±0.1	0.043±0.022	20±9

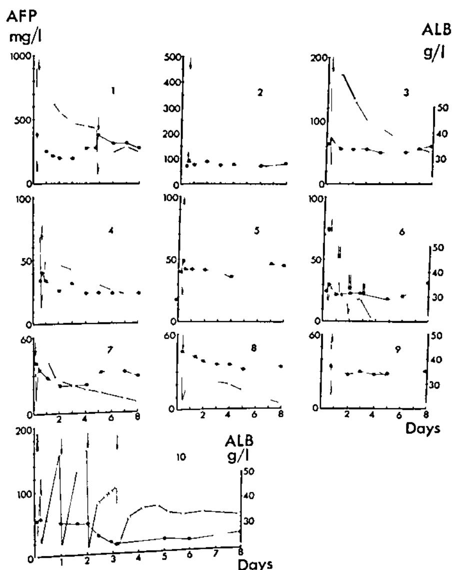


Fig. 2. Serum AFP and albumin concentrations in 10 newborns of Group II (Table 1) during and after blood exchange transfusion(s). Blood exchange transfusions are indicated by arrows. Time 0 means 8 a.m. on the day of the first transfusion. O=AFP ●=albumin

Table 3 *Increase in the total amount of AFP in two patients*

Patient	Time from beginning of blood exchange transfusion	Hcr	Plasma volume (ml)	AFP conc in plasma (mg/l)	Amount of AFP (mg)			
					Intra vascular	Extra vascular	Total	Removed during transfusion
Case 1 Group I	0 min	0 45	143	102	14 6[a]	29 2[b]	43 8	16 7[a]
	55 min	0 46	141	13	1 8[a]	25 3	27 1[c]	
	24 h	0 43	148	78	11 5[a]	23 0[b]	34 5	
Case 4 Group II	0 min	0 42	114	69	7 9[a]	15 8[b]	23 7	11 8[a]
	40 min	0 48	102	13	1 3[a]	10 6	11 9[c]	
	9 h	0 46	106	50	5 3[a]	10 6[b]	15 9	

[a] Obtained by measurement
[b] 2 0×intravascular amount
Total amount of AFP in the beginning of exchange minus the amount removed during transfusion

Changes after the transfusion

The initial albumin level correlated positively with gestational age ($r=0\ 63$, $p<0\ 05$) (Fig 2) During the 3–5 days after the transfusion the mean albumin concentration decreased from 37 g/l (range 27–40 g/l) to 31 g/l (23–38 g/l), i e a mean fall of 14% The correlation between the decrease in albumin concentration and the birthweight was negative ($r=-0\ 69$, $p<0\ 05$) consequently final albumin levels were lowest in low birthweight babies ($r=0\ 76$ $p<0\ 01$) The low albumin level can be attributed to inadequate or lacking albumin synthesis in the low birthweight babies The decrease in albumin concentration was similar in all aetiological groups (when standardized for birthweight)

Unlike albumin the plasma AFP concentration—after the decrease caused by transfusion—returned to a level of about 80% of the initial concentration in 1 day This rapid increase in AFP might be due to a new equilibrium between the intra and extravascular AFP to the synthesis of new AFP or to both During the transfusion 92–95% of the intravascular protein is exchanged (19) After a sudden depletion of the intravascular protein a new equilibrium between the intra and extravascular protein pools should be reached in about 1 day (19) Assuming that no shift from the extravascular pool to the circulation occurs during transfusion and that the ratio of AFP in the extravascular pool to that in the circulation will again reach the value of 2 0 (4, 26) after transfusion the predicted concentration of plasma AFP in the new equilibrium is about 68% of the initial value The concentrations measured were clearly higher (Table 1)

In multiple transfusions the expected concentration of plasma AFP in the new equilibrium should be about 46% after two transfusions at 24-hour intervals, 10% after 6 transfusions and 3% after 9 transfusions The concentrations observed were 32% (case 1 2 transfusions) 40% (case 10, 6 transfusions), and 26% (case 6 9 transfusions) of the original, respectively (Fig 2) In cases 6 and 10, the transfusions took place in four clusters of 2–3 transfusions As the intervals between the clusters were approximately 24 hours, equilibrium was probably reached four times, resulting in a theoretical final concentration of 21% of the initial The values obtained, 40% and 26%, strongly suggest synthesis of AFP

Table 3 shows the results obtained in two infants whose total amount of AFP increased by 4 0 mg in 9 hours (7 μg/min) and 7 4 mg in 24 hours (5 μg/min) The increase was slightly less than that produced by isolated perfused human foetal liver at 14–20 weeks of gestation (19–26 μg/min) (10) Assuming that the blood volume of the infant remained unchanged, the approximate rate of AFP synthesis was 2–4 mg/kg/day

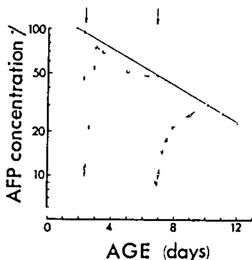


Fig 3 Serum AFP concentrations as percentages of the initial level in case I of Group II (see Fig 2) Arrows indicate blood exchange transfusions The AFP concentration before the first transfusion is taken as 100%

After the rapid increase the plasma AFP level changed to a slow decline This decrease was exponential and apparently not much affected by losses of AFP up to 50 mg (mean 18 mg) during the transfusions Fig 3 shows the values for one infant and the results were essentially the same for the other patients

DISCUSSION

Because of the great difference in plasma AFP concentration between newborns and donors, blood exchange transfusions seemed suitable for measurement of the disappearance rate of endogenous AFP The infants in this study were not normal, as they had neonatal liver insufficiency or isoimmunization The results are probably applicable even to normal infants, as the turnover rate of endogenous AFP and its rapid increase in serum after the transfusions in this series did not depend on the aetiology of hyperbilirubinaemia, gestational age, birthweight or age of the baby during the transfusions

The theoretical curve based on equation (2) did not fit well with the concentrations of AFP observed during the transfusions At the beginning of the transfusions the theoretical AFP concentrations calculated from the λ values were lower than those actually measured, towards the end the reverse was true The method of calculating the turnover rate (λ) of endogenous AFP assumes that the AFP concentration in the extravascular space does not change during the transfusion This requirement was not met, however, since AFP diffuses from the extravascular compartment to the intravascular pool In consequence the λ-values are too high, one can approximate that the actual values may be only one third as high This was especially true when the transfusions were slow Therefore, as the actual turnover rate of AFP could not be measured, we estimated only its magnitude The half life of AFP was a few hours in contrast to the few days calculated from the decrease in plasma AFP concentration The slow decrease in plasma AFP concentration associated with a rapid turnover rate suggests continuous synthesis of AFP after birth

The plasma concentration of albumin was higher in the high birthweight group, as shown earlier by Bergstrand et al (1) The mean albumin level decreased from 37 to 31 g/l within the first 3–5 days after transfusion This decrease was very similar to that found by Karlsson et al (9) in infants without transfusions It appears that albumin concentration is not affected by the transfusions

The plasma AFP concentration was expected to return to about 68% of the initial level after the transfusions The calculations were based on the assumption that during transfusion the extravascular pool remains untouched However part of the AFP in the extravascular compartment will diffuse into the circulation during the transfusion which makes the expected 68% level an overestimate In spite of this the concentration of AFP greatly exceeded expectation in all cases The significant increase of plasma AFP concentration after transfusions strongly suggests postnatal synthesis of AFP, although there remains a conceivable possibility that it might be attributable to a decrease in the turnover rate of AFP

The age of the infant being transfused did not influence the turnover rate of AFP, which means that the turnover rate remains roughly constant during the first few days of life The decrease in the plasma AFP concentration was exponential (Fig. 3), which implies that the synthesis of AFP also decreases exponentially, as suggested earlier (5)

The results of this study can be summarized as follows

1) The endogenous turnover rate of AFP is about 0 04 min^{-1}, giving a half life of about 20 min

2) The decrease in plasma AFP concentration is much slower, which suggests that synthesis of AFP continues after birth

3) Plasma AFP concentration decreases exponentially after birth, but a sudden depletion of AFP caused by blood exchange transfusion is rapidly restored by increased production of AFP It is postulated that the serum AFP concentration controls the synthesis of AFP via a negative feedback mechanism

4) In contrast to animals, there is no evidence that adult human blood contains a factor which inhibits the synthesis of AFP in neonates

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Submitted Feb 9 1979
Accepted Dec 14 1979

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Acta Pædiatr Scand 69 499-503, 1980

UROPATHIES DIAGNOSED IN THE NEONATAL PERIOD: SYMPTOMATOLOGY AND COURSE

A Report of 40 Cases

A BENSMAN, J J BAUDON, J P JABLONSKI and G LASFARGUES

From the Chair

ABSTRACT Bensman, A , Baudon, J J , Jablonski, J P, and Lasfargues, G (Chaire ... Service de Chirurgie Viscerale, Faculté de Medecine

1980.—Forty infants with a uropathy diagnosed during the ... studied. Presenting signs were urinary tract infection in one-half of the cases, disorders of micturition, pelvic or abdominal wall malformations, abdomino-pelvic mass, and macroscopic hematuria Obstructive uropathy was observed in 17 children and vesicoureteric reflux in 29. We noted a high incidence of extrarenal malformations (14 of the 40 cases) in this series of uropathies diagnosed during the neonatal period Despite early diagnosis, the course was not favorable in 11 cases with congenital anomalies of the renal parenchyma

KEY WORDS Neonatal uropathy, urinary tract infection

The severity of a uropathy depends on the degree of renal involvement with which it is often associated It is generally accepted that the renal involvement is due to three main mechanisms

1 Congenital renal lesions, principally dysplasia

2 Increased pressure in the excretory tract in obstructive uropathy

3 Lesions due to interstitial *nephritis in* pyelonephritis

The last two factors can be reduced or even eliminated by early nephro urological treatment, hence it is important that the diagnosis is made as early as possible

We made a retrospective study of a series of infants in whom uropathies were diagnosed during the first two months of life The aim of this study is to describe

1 Their familial history
2 The presenting signs at diagnosis
3 The types of uropathy diagnosed.
4 Associated malformations
5 The course

PATIENTS AND METHODS

Forty infants were studied, 29 males and 11 females *Uropathy was defined* as the existence of ureteropyelocalyceal anomaly on IVP *Thus, this* study excludes *infants* with reflux into a non-dilated ureter and with a *normal IVP* Diagnosis of uropathy was made during the first 8 *weeks of life (Fig 1)* in all the cases studied, 34 diagnoses were made during the first 4 *weeks of life,* of which 18 were during the first week

All infants underwent IVP and retrograde cystography Urinary infection was defined as bacteriuria $>10^5$ organisms/ml Glomerular filtration was studied by endogenous *creatinine* clearance and maximum urinary *concentration* ... Vesicoureteric ... e I—incomplete ... de II—complete reflux into the entire *ureter without* distending it, grade III—complete *reflux into a distended* ureter, grade IV—*complete reflux* with marked distension of the ureter and renal pelvis

RESULTS

1. *Familial history*

No familial uropathy was found In 30 cases pregnancy was uncomplicated, in three hypertension occurred, and in seven cases *no information* was available *Vertex* delivery oc-

P R Ferritin turnover in plasma an opportunistic use of blood removed during exchange transfusion *Pediatr Res 9* 127 1975
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Submitted Feb 9 1979
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Table 1 *Length of follow up of the infants*

Length of follow up	Number of infants
3 months to 1 year	18 (2 deaths)
1 to 2 years	8
2 to 3 years	5
3 to 6 years	5
6 to 15 years	4

Complex uropathies (3 cases) Two cases of functionless kidney manifested by an abdominal mass above ureteral atresia Pathological studies showed multicystic dysplastic lesions

One case of moderate dilatation of the ureter and pelvis associated with bladder extrophy

4 *Extrarenal malformations*

Extrarenal malformations occur frequently and were found in 14 children in our series In 5 of our cases they were overt manifestations of the uropathy In the other cases they were sought for and found in 9 infants Each of the following malformations was found once, some children presented several malformations

(a) *Malformations of the gastrointestinal tract* volvulus of the small intestine, atresia of the bile ductules, stenosis of the pylorus gastro oesophageal reflux causing oesophagitis imperforate anus

(b) *Genital malformations* hermaphroditism with ovotestis, hydrocolpos, vaginal atresia with bicornuate uterus, unilateral absence of an ovary with impermeable uterine tube bilateral undescended testes

(c) *Skeletal malformations* rachischisis of several vertebrae sacral malformation or lumbarisation congenital hip dislocation, clubfoot

(d) *Cardiac malformations* pulmonary stenosis ductus arteriosis, ventricular septal defect

(e) *Facial malformations* complex dysmorphy harelip

(f) *Muscular malformations* absence of the abdominal muscles, bladder extrophy, exomphalos and middle umbilical fold

5 *The course of the uropathies*

The course of the uropathies was followed for varying lengths of time (Table 1) Four groups of infants can be distinguished

Group I Twelve infants with a favorable course Creatinine clearance is above 80 ml/min/1 73 m² and their urological status is excellent

Eight of these 12 infants had two normal kidneys under radiological examination Maximum urinary concentrating capacity was performed in 5 cases and was always normal (maximum urinary osmolality above 850 mOsm/kg) Initial uropathy was grade III vesicoureteric reflux

The other four of these 12 infants had one normal kidney by radiological examination and discrete lesions in the contralateral kidney (calyceal clubbing) Maximum urinary concentrating capacity was normal The initial uropathy in one case was grade III vesicoureteric reflux and in three cases pelvi ureteric obstruction

Group II Seven infants with a favorable course, based on normal glomerular filtration and maximum urinary concentrating capacity However, only one kidney was functioning with normal radiographic appearance

In five cases, nephrectomy was performed Three of these kidneys were small and functionless, and associated with massive vesico ureteric reflux (grade IV) Two of the kidneys were large, with a nodular appearance which histological examination showed to be multicystic renal dysplasia

In the two other cases a kidney with impaired function was left in situ The initial uropathies were grade IV vesicoureteric reflux in one case and pelvi ureteric obstruction in the other

Group III Ten infants with normal glomerular filtration but with reduced maximum urinary concentrating capacity All these kidneys showed anomalies on radiological examination i e , calyceal clubbing and loss of renal parenchyma The initial uropathy was pos

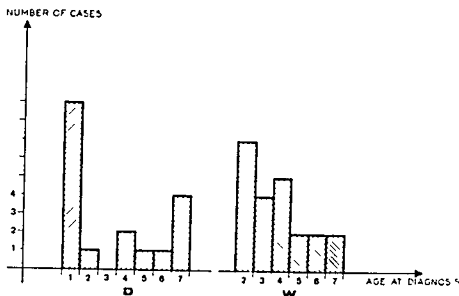


Fig 1 Number of cases and age at diagnosis

curred in 34 cases, breech delivery in 3, and delivery by cesarean section in 4 cases these cesarean sections were performed because of fetal distress in 2 cases, hypertension in 1, and contracted pelvis in 1 No oligohydramnios was noted In 27 cases, delivery was at term and in 7 premature Information was not available in 6 cases The infant's state at birth was normal in 37 cases, transient respiratory distress was noted in 1 case, and discrete neurological signs possibly indicative of fetal distress were present in 2 cases

2 *Presenting signs*

Five types of presenting signs were noted

1) Urinary infection (21 cases), 2) disorders of micturition (7 cases), 3) abdomino-pelvic mass (6 cases), 4) macroscopic hematuria (1 case), 5) pelvic or abdominal wall malformation (5 cases) which led to urological investigation i e , absence of the abdominal muscles, bladder extrophy, sexual ambiguity with hypospadias, imperforate anus, and lipoma with meningocele

3 *Types of uropathy*

Four types of uropathy were discovered (more than one type could be present in each child)

Obstructive uropathy (17 cases) 9 posterior urethral valves, 5 pelvi ureteric obstructions, of which 2 were bilateral, 2 obstructions of the ureterovesical junction, 1 bladder neck obstruction

Duplication of the ureter (6 cases) in 2 cases bifid ureter only with vesicoureteric reflux, in 4 cases a complete ureteric duplication associated in 3 cases with grade III vesicoureteric reflux into the inferior pelvis, in 1 case a ureterocele of the upper pole ureter of a duplex kidney

Vesicoureteric reflux (29 cases) Due to bladder outflow obstruction in 8 cases bladder neck obstruction in one case and posterior urethral valves in 7 cases After treatment of the obstruction, vesicoureteric reflux disappeared in 3 cases, persisted in 5 cases and required antireflux surgery in 3 cases

There was no bladder outflow obstruction in 21 cases

Grade III vesicoureteric reflux in 9 cases 5 showed spontaneous remission, in 4 other cases, the follow up of less than 6 months is insufficient

Grade IV vesicoureteric reflux in 12 cases All these infants underwent surgery

3 nephrectomies for massive reflux into a small, functionless kidney

7 operative cures of reflux,

2 cutaneous ureterostomies, one for Prune-Belly syndrome and the other for massive dilatation of the entire excretory tract without bladder outflow obstruction and in which operative cure had failed

However, in 23 infants in whom follow up was longer than 6 months, surgery was required 14 times despite the disappearance of the urinary tract infection This is an illustration of the high frequency of vesicoureteric reflux due to malformation in our series Associated malformations occurred frequently, in 14 of the 40 infants (34%) It is well known that uropathies are often associated with anorectal anomalies (2) spina bifida, agenesis of the abdominal muscles, cardiac malformations (9 12) and with malformations of the gastrointestinal tract Other types of malformations which are more frequently associated with uropathies were not found in our series, such as chromosomal aberrations (7, 8, 12), ear deformities (6), or a single umbilical artery (5) The incidence of these anomalies is, however variable from one series to another Bois (1) studying 436 cases of uropathy, found 14% of non urological malformations associated with malformations of the upper urinary tract and 9 8% associated with lower urinary tract malformations On the other hand Coulton (3) while studying 318 infant autopsies with known non urological congenital anomalies found 8 2% with associated urological anomalies

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Submitted Aug 7 1979
Accepted Nov 12 1979

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Table 2 *Urological and nephrological status of 8 infants with an unfavorable course*

Glomerular filtration (ml/min/1 73 m²)	Significant I V P lesions	Single kidney	Initial uropathy
31	×		Mega ureter+reflux (M R)
35	×	×	P U V (Posterior urethral valves)
40	×		M R
45		×	Bladder neck obstruction
48	×		P U V
60			Bladder exstrophy
75	×	×	M R
75			M R

terior urethral valves in 6 cases and grade-IV vesicoureteric reflux in four cases

Group IV Eleven infants had an unfavorable course

Two of these infants died, one of acute renal failure in the neonatal period due to a single functionless polycystic kidney The other died of infection

One infant, 15 months of age, is being treated by hemodialysis The initial uropathy was due to posterior urethral valves At birth creatinine clearance was below 10 ml/min/1 73 m² and cutaneous ureterostomy improved glomerular filtration only slightly

Eight infants had glomerular filtration rates below or equal to 75 ml/min/1 73 m² (Table 2) In five cases, renal failure was associated with significant radiological lesions, i e , calyceal clubbing, significant loss of renal parenchyma In three cases this occurred in a single kidney

DISCUSSION

Uropathies are rarely diagnosed in the neonatal period, although to our knowledge there are no statistical data concerning their actual occurrence The classical method is to seek uropathy particularly in cases with a familial history of obstructive uropathy (11) or in cases with an abnormal volume of amniotic fluid, which could reflect a disorder of fetal diuresis (10) In our study we found no such cases, which at any rate appear rare (9) It is possible, however, that the volume of amniotic fluid was not noted in all cases The predominence of males (70% of our cases) is well known, particularly as a result of lower tract obstructions

The discovery of urinary infection led to the early diagnosis of uropathy in 21 out of 40 cases In a personal series of 60 cases of neonatal urinary tract infections, as well as in other series in the literature, uropathies were found in only 5 to 15% of cases (4) However, in our experience one out of two neonatal uropathies was revealed by a urinary tract infection

Obstructive and severe uropathy was found in 17 of the 40 infants (42%)

Despite early diagnosis of the uropathy, the course was not favorable in 11 of the 40 infants studied (27%) These children had congenital anomalies of the renal parenchyma

This group includes two deaths and one child whose follow-up was less than one year The other children with unfavorable courses have been followed up for more than one year Of the children studied, 18 had a short follow up period At the end of this period 15 of these children have normal glomerular filtration rates

Eight of the 40 infants underwent nephrectomy Renal dysplasia (12) was found in each case, confirming the incidence of congenital anomalies of the renal parenchyma in severe uropathies

Of the 40 infants 29 (75%) had vesicoureteric reflux The urinary infection itself could explain the vesicoureteric reflux (13) and the dilatation of the ureter and pelvis

Acta Pædiatr Scand 69 505–510, 1980

CIRCULATING ANTIDIURETIC HORMONE DURING LABOUR AND IN THE NEWBORN

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ABSTRACT. Leung, A , McArthur, R , McMillan, D , Ko, D , Deacon, J , Parboosingh, J and Lederis K (the Divisions of Paediatrics, Pharmacology and Obstetrics, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada) Circulating antidiuretic hormone during labour and in the newborn Acta Paediatr Scand, 69 505, 1980.—Using a high specificity radioimmunoassay, antidiuretic hormone (ADH) concentrations were measured in the plasma of 33 expectant mothers during labour, in cord arterial and venous plasma of their infants at the time of delivery (19 delivered vaginally, 14 delivered by Cesarean section) and in the plasma of the same infants in the first few days of life

during the first day of life Stressed babies and babies subjected to difficult deliveries had higher plasma levels of ADH

KEY WORDS Antidiuretic hormone, labour, newborn, stress

he development of accurate radioimmuno-ssay methods for the measurement of anti-liuretic hormone (ADH) and other posterior pituitary hormones (3) has opened an impor-ant area for clinical investigation in perinato-logy Although fetal and cord blood ADH levels are known to be high during parturition (4 6 8) the mechanism, importance and post natal duration of ADH excess are not known Hadeed and associates (8) report that cord arterial ADH concentrations are high at birth and fall to normal during the first day of life These authors suggest that cerebral compres-sion from vaginal delivery, rather than anoxia, is the stimulus for ADH hypersecretion Stressed infants (e g pneumonia, respiratory distress syndrome infections) have been re-ported to be hyponatremic with high ADH levels (10 13 18), but documentation has been confined for the most part, to individual case reports The purpose of our study was to in-vestigate maternal, cord, and post natal plasma ADH levels in the human newborn

PATIENTS AND METHODS

A total of 33 mothers and their infants were studied Group A consisted of 14 infants delivered by Cesarean section and Group B of 19 infants delivered vaginally

In Group A, the age of the mother ranged from 22 to 33 years with a mean of 27±3 6 years The mothers varied from gravida (G) 1 Para (P) 0 to G4 P2 Nine were delivered by elective Cesarean section and five by emergency Cesarean section because of fetal distress and/or poor progression of labour In this group there were ten male infants and four female infants All were term infants with a mean birth weight of 3 553 g

In Group B the age range of the mother was from 18 to 35 years with a mean of 26 8±5 6 years These mothers ranged from G1 P0 to G3 P2 Fifteen infants were born by normal spontaneous delivery and four were delivered using forceps There were eleven male infants and eight female infants Sixteen infants were term and three were premature (delivery before 37 weeks), their mean birth weights being 3 451 g and 1 535 g respectively

Informed written consent was obtained from all mothers Five ml venous blood was obtained from each of the mothers during the first stage of labour Ten ml blood was obtained from each infant s cord umbilical vein and three ml from the cord umbilical arteries as soon as the infant was born Subsequently three ml blood was taken from the infant within the first 24 hours and once again at a convenient hour of the day, within the first five days of post natal life

All blood samples were collected in heparinised plastic

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ABSTRACT Leung, A , McArthur, R , McMillan, D , Ko, D , Deacon, J , Parboosingh, J. and Lederis K. (the Divisions of Paediatrics, Pharmacology and Obstetrics, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada). Circulating antidiuretic hormone during labour and in the newborn. Acta Paediatr Scand, 69, 505, 1980.—Using a high specificity radioimmunoassay, antidiuretic hormone (ADH) concentrations were measured in the plasma of 33 expectant mothers during labour, in cord arterial and venous plasma of their infants at the time of delivery (19 delivered vaginally, 14 delivered by Cesarean section) and in the plasma of the same infants in the first few days of life. Extremely high concentrations of ADH (about 50 times higher than adult basal concentrations) were present in cord arterial blood indicating active fetal production of ADH Plasma ADH decreased rapidly within an hour after birth and usually fell to adult basal levels during the first day of life Stressed babies and babies subjected to difficult deliveries had higher plasma levels of ADH

KEY WORDS Antidiuretic hormone, labour, newborn, stress

The development of accurate radioimmunoassay methods for the measurement of antidiuretic hormone (ADH) and other posterior pituitary hormones (3) has opened an important area for clinical investigation in perinatology Although fetal and cord blood ADH levels are known to be high during parturition (4, 6, 8) the mechanism, importance and postnatal duration of ADH excess are not known Hadeed and associates (8) report that cord arterial ADH concentrations are high at birth and fall to normal during the first day of life These authors suggest that cerebral compression from vaginal delivery, rather than anoxia, is the stimulus for ADH hypersecretion Stressed infants (e g pneumonia, respiratory distress syndrome, infections) have been reported to be hyponatremic with high ADH levels (10 13, 18), but documentation has been confined, for the most part, to individual case reports The purpose of our study was to investigate maternal, cord, and post-natal plasma ADH levels in the human newborn

PATIENTS AND METHODS

[illegible] 33 years with a mean of 27+3 6 years The mothers varied from gravida (G) 1 Para (P) 0 to G4 P2 Nine were delivered by elective Cesarean section and five by emergency Cesarean section because of fetal distress and/or poor progression of labour In this group, there were ten male infants and four female infants All were term infants with a mean birth weight of 3 553 g

In Group B the age range of the mother was from 18 to 35 years with a mean of 26 8±5 6 years These mothers ranged from G1 P0 to G3 P2 Fifteen infants were born by normal spontaneous delivery and four were delivered using forceps There were eleven male infants and eight female infants Sixteen infants were term and three were premature (delivery before 37 weeks) their mean birth weights being 3 451 g and 1 535 g respectively

Informed, written consent was obtained from all mothers Five ml venous blood was obtained from each of the mothers during the first stage of labour Ten ml blood was obtained from each infant s cord umbilical vein and three ml from the cord umbilical arteries as soon as the infant was born Subsequently, three ml blood was taken from the infant within the first 24 hours and once again at a convenient hour of the day, within the first five days of post natal life

All blood samples were collected in heparinised plastic

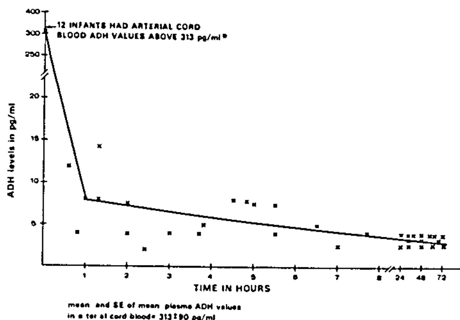


Fig 1 ADH levels in arterial cord blood and during early postnatal life A mean ADH concentration in arterial cord blood was calculated from 33 infants The levels illustrated during the post natal period represent individual measurements

tubes on ice centrifuged and the plasma kept frozen at −20°C until assay Assay of antidiuretic hormone (ADH) was determined by a high specificity radioimmunoassay for vasopressin developed in our laboratory (2, 9) Cord arterial blood pH hematocrit and protein were measured in all cases

RESULTS

Comparison between different groups of infants are shown in Table I The data were analysed by "Student's" test (15), a $p<0.05$ being taken as indicative of significance

Maternal plasma ADH levels during labour ranged from 1 94 to 7 53 pg/ml with a mean of 4 14 pg/ml

Serial measurements of ADH levels at birth and during the first 5 days of post natal life are shown in Fig 1 Mean cord arterial blood level of ADH of these 33 patients was 313 pg/ml The l

life (<

basal levels during the first day of life

Great variation was observed in the ADH levels in the cord arterial and venous blood samples of individual infants Cord arterial ADH concentrations varied from 3 5 pg/ml to >2 000 pg/ml, and cord venous ADH concentrations ranged from 1 94 pg/ml to 354 pg/ml However, in spite of the great individual variation, the concentration of ADH in arterial cord blood was significantly higher than that in cord venous blood (Fig 2) These differences were similar in infants delivered by Cesarean section or vaginally (see Table 1)

Of the fourteen infants delivered by Cesarean section, mean ADH levels in cord arterial blood were significantly higher in those cases in which this was carried out because of clinical evidence of fetal distress (Fig 3)

Cord arterial ADH levels appeared to be

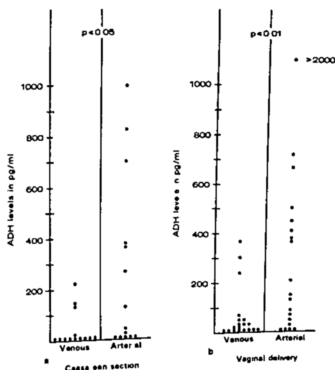


Fig 2 ADH levels in cord arterial and venous blood following (*a*) Cesarean section and (*b*) vaginal delivery

Table 1 *Cord arterial and venous ADH concentrations related to method of delivery, presence of absence of fetal distress, and length of gestation*

Groups of infants	*n*	Cord plasma ADH in pg/ml			
		Arterial		Venous	
		Range	Mean ± SE	Range	Mean ± S E
I *Mode of delivery*					
Group A					
Cesarean section	14	3 58–1000	236±92	1 94–227	46±21
1 Elective Cesarean section	9	3 58–375	72±45	1 94–21	6 3±2 2
2 Cesarean section because of fetal distress	5	272–1000	637+138	4 1–227	79±45
Group B					
Vaginal delivery	19	5 6–>2000	375±122	1 94–354	74±28
1 Normal spontaneous delivery	15	8 33–>2000	454+143	4 2–354	68±30
2 Forceps delivery	4	5 5–121	60±24	1 94–308	80±75
II *Presence or absence of fetal distress*					
1 Infants with fetal distress	13	8 33–>2000	573±40	1 94–354	92±130
2 Infants with no fetal distress	20	3 58–666	168±15	1 94–245	40±63
III *Length of gestation*					
1 Term infants (excluding those with fetal distress)	20	1–160*	48±15*	1–100*	40±60*
2 Premature infants	3	51–313*	151±81*	2–210*	94±130*

* Expressed in pg/kg/ml

lower in infants delivered by outlet forceps as compared with those born by normal vaginal delivery (Fig 4) The mean duration of the second stage of labour of the forceps assisted deliveries was 52±15 minutes, whereas the mean duration of the second stage of non-forceps assisted deliveries was 16 8±12 minutes

As can be seen in Table 1, in premature infants, of which there were only 3 in our study, ADH levels in cord arterial blood seemed to be higher than in term infants when the results were expressed in relation to body weight Also, infants delivered by either Cesarean section or vaginal delivery suffering fetal distress had higher cord arterial blood ADH levels than did infants who did not suffer fetal distress The former group of infants included those with clinical evidence of fetal distress (variable deceleration and/or meconium staining) Two infants of diabetic mothers and one infant with a single ventricle and atrium also had high cord arterial blood ADH levels The clinical course of all infants, except for the patient with a single ventricle and atrium, was uncomplicated

The ADH levels in the cord arterial blood did not differ between male and female infants, nor between smoking and non-smoking mothers, or between those in whom labour was medically induced or spontaneous Cord blood pH was >7 25 in 32 of 33 patients and cord hematocrit and total protein were normal in all instances

Significance of differences (Table 1)

1 ADH concentrations were higher in umbilical arterial than venous blood ($n=33$, $p<0\ 05$).

2 ADH concentrations in umbilical arterial blood were higher in those infants with Cesarean section done because of clinical evidence of fetal distress ($n=5$, $p<0.005$) compared with those born by elective Cesarean section.

3. ADH concentrations in umbilical arterial blood were lower in infants delivered by forceps ($n=4$, $p=0\ 05$) than in those born by normal vaginal delivery.

4. Infants suffering fetal distress had higher

Fig. 3. ADH levels in arterial cord blood following (*a*) elective Cesarean section and (*b*) Cesarean section performed because of fetal distress.

Fig. 4. ADH levels in arterial cord blood following (*a*) delivery by outlet forceps and (*b*) delivery by normal spontaneous delivery.

umbilical arterial ADH concentrations ($n=13$, $p<0.05$).

5. Premature infants had higher umbilical arterial concentrations ($n=3$, $p=0.02$) than term infants.

DISCUSSION

Several investigations in both humans and animals have shown that fetal ADH levels measured by a bioassay method rise just prior to the onset of labour and are markedly elevated during labour (4, 6, 16). Extremely high concentrations of ADH (>100 pg/ml) were found at the time of birth in 18 infants investigated in the present study. Earlier studies in sheep have shown clearly that the fetus is autonomous with respect to ADH secretion and that the placenta is impermeable to ADH (1). Our findings demonstrating increased ADH concentrations in cord arterial blood compared to cord venous and maternal blood, indicate that fetal production of ADH also occurs in the human at the time of delivery. Our findings support the hypothesis of Hadeed et al. (8) that the "cerebral squeeze" resulting from labour and the birth process stimulates hypersecretion of ADH in the fetus. However other mechanisms which might result in ADH secretion (e.g. blood volume, osmolality or P_{O_2} changes occurring in the fetus during uterine contractions) may also play a role in the production of elevated cord arterial ADH levels.

Following delivery, infant plasma ADH values feel rapidly reaching close to adult basal levels by 2–4 hours of age. This may be related to resorption of lung fluid and placental transfusion of blood which increases circulating blood volume at the time of birth. Loss of body weight in the normal newborn infant in the first days of life may be related to fall in

ADH concentration and subsequent excretion of excessive fluid

Asphyxiated infants and infants with fetal distress release increased amounts of ADH (5, 7, 8, 11, 12). In our study, infants born by Cesarean section because of fetal distress had higher ADH levels in arterial cord blood than infants born by elective Cesarean section. The normal cord arterial blood gases indicate that intervention was accomplished before the distress became severe. It is probable that both trial labour and fetal distress resulted in increased ADH levels in those infants born by emergency Cesarean section.

Premature infants and infants in distress apparently had higher cord arterial ADH levels than term infants or infants who did not have fetal distress. Regardless of whether or not ADH concentrations in the premature are ;her than, or equal to, the levels found in rmal term infants, it is evident that, in the emature, the mechanism by which ADH is oduced and secreted is intact. Because pre-ature and stressed infants may require intra :nous therapy when born, and because the ılf life of ADH in the newborn is not known, ıch babies may be at risk for water intoxica on if careful monitoring of appropriate fluid ıerapy is not provided.

Infants delivered by forceps had lower ADH :vels in the cord arteries than those not elivered with forceps. This may be due to the rotection effect of forceps on the fetal head. On the other hand, the duration of the second stage of labour in forceps deliveries was considerably longer. Since no information is available on the pituitary reserve of ADH during delivery, the possibility cannot be excluded that the easily releasable pool of ADH (14) may be exhausted during a prolonged birth process.

The wide variation in the ADH content in individual infants may be due to variations in the degree of stress during labour and birth. However, it is tempting to suggest that wide scatter of values may reflect an artificial variation. both th- [illegible] oduc-ing neurones have been shown to exhibit activity in bursts; release of hormones in spurts is most likely related to such bursts. Any single measurement of ADH in blood, therefore, has to be interpreted with due regard to such secretory bursts (17). Considerably larger numbers of patients may have to be analyzed in order to draw unequivocal conclusions.

ACKNOWLEDGEMENTS

The authors are grateful to Mr Joseph Yip for help in statistical analyses and to Mrs Joyce Cunningham for expert secretarial assistance.

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Submitted Sept 21, 1979
Accepted Nov 27, 1979

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Acta Pædiatr Scand 69 511–516, 1980

PPD TESTING AS A DIAGNOSTIC AID IN NON-TUBERCULOUS MYCOBACTERIOSIS

Clinical and Immunological Investigations in 4 Children with Cervical Lymphadenitis

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From the Department of Paediatrics Malmo Allmanna Sjukhus Malmo and the Department of Medical Microbiology University of Lund Lund Sweden

ABSTRACT Hallberg, A , Hallb
Malmo Allmanna Sjukhus, and D
Lund, Sweden) PPD testing as a c
and immunological investigations in 4 children with cervical lymphadenitis Acta Paediatr Scand, 69 511, 1980.—Four children suffering from unilateral cervical lymphadenitis with histopathological changes typical of mycobacteriosis were seen during a short time None of the Children had been BCG vaccinated Mycobacteria belonging to the *Myobacterium avium-intracellulare* complex were isolated from excised lymph nodes in two of the patients Intracutaneous tests with PPD from *M tuberculosis* were negative in all the children, whereas two children responded to each of 3 PPDs prepared from atypical mycobacteria Two patients were unreactive in all the skin tests Lymphocyte transformation tests in vitro with a battery of various PPDs indicated sensitization to atypical mycobacteria in two children, one of which was negative in the skin tests All the patients had normal plasma Ig concentrations but two patients had low proportions of T lymphocytes in the peripheral blood One of these also had reduced total numbers of T cells Nevertheless, lymphocyte responses in vitro to phytohaemagglutinin were normal in all the children The results show that cutaneous and in vitro tests with a battery of different PPDs have a place as diagnostic adjuncts in atypical mycobacteriosis We suggest that immunological competence is analysed in such patients

KEY WORDS Mycobacteria, lymphadenitis, T lymphocytes, immunodeficiency

In Sweden the regular BCG vaccination of neonates was abandoned in April 1975, because of an increasing incidence of side-effects Since then reports have appeared in this country (24) of cervical adenitis, caused by mycobacteria other than *Mycobacterium tuberculosis* or *Mycobacterium bovis* Probably, these infections with so-called 'atypical mycobacteria' are now more common, but the reason for this is unclear Cessation of the general BCG vaccination may be a major factor, since immunity to BCG protects not only against *M tuberculosis* and *M bovis* but also against some atypical mycobacteria (9) Also, some individuals may have an increased susceptibility to atypical mycobacteria In patients with immunodefic····· or other debilitating diseases these bacteria may cause generalized infections (17, 20, 22)

We therefore investigated 4 children, 18–34 months old and not BCG vaccinated, with cervical adenitis of proven (2 patients) or probable non tuberculous mycobacterial origin for their immunological competence and studied their response in the skin and in vitro to PPD from various mycobacteria

METHODS

Haematological data were obtained with routine laboratory procedures Plasma IgG, IgA and IgM were determined by electroimmunoassay (13) BCG vaccinated healthy adults were the controls in the *in vitro* lymphocyte tests

Lymphocyte surface markers Lymphocytes were prepared from freshly collected heparinized blood and T

Table 1 *Clinical data*

C=cycloserine, E=ethambutol I=isoniazid R=rifampicin

Patient	Sex	Age (mo)	Affected lymph nodes	Culture for mycobacteria	Treatment
H H	M	18	Left sub-mandibular	Negative	Excision
J T	M	21	Left sub-mandibular	*M avium intracellulare*	Excision + chemotherapy (I+E+C)
J A	F	22	Left lower cervical	Not done	Excision + chemotherapy (R+I)
M M	F	34	Left sub-mandibular	*M avium intracellulare*	Excision + chemotherapy (I+E+C)

lymphocytes demonstrated by rosetting with untreated sheep erythrocytes (SRBC) as described elsewhere (11) In one child (MM) lymphocytes were analysed also for receptors for Fc of IgG receptors for complement and surface membrane IgM with methods described previously (10) Surface membrane IgM was demonstrated by direct immunofluorescence using F(ab)$_2$ fragments of FITC conjugated goat anti human IgM (Kallestad) as reagent

Activation of lymphocytes with phytohaemagglutinin (PHA) Lymphocytes were prepared as described previously (11) but the final purification by centrifugation on Isopaque Ficoll was omitted The cells were suspended in Gibco RPMI 1640 with 20 mM HEPES buffer 0 4% human serum albumin and phytohaemagglutinin (Burroughs Wellcome) The suspension was distributed in quadruplicate in tissue culture plates 10^5 cells per well and incubated at 37°C for 72 hours During the last 4 hours of cultivation 1 μCi of tritiated thymidine (TRK 120 RCC Amersham England) was included in the tissue culture medium The cells were deposited onto glass fibre filters and the adsorbed activity determined by liquid scintillation

Activation of lymphocytes with PPDs Lymphocytes were cultivated in the presence of PPD prepared from the following mycobacteria *M tuberculosis M kansasii* RS 30 *M scrofulaceum (M marianum)* RS 95 *M intracellulare* RS 23 and *M fortuitum* RS 20 All the preparations were obtained preservative free from Statens Seruminstitut Copenhagen and were used at 10 μg/ml The experimental protocol closely followed that for activation with PHA but the tissue culture medium was supplemented with 15% foetal bovine serum instead of human serum albumin The cultivation period was extended to 120 hours Statistical analysis of the results was performed on ^{10}log transformed cpm values Mean standard error of quadruplicate log cpm determinations was 0 11 (range 0 03–0 23) Ratios were also calculated between mean cpm (not log transformed) in the presence of atypical PPD and PPD from *M tuberculosis*

Skin tests Purified tuberculin from *M tuberculosis* (1TU/0 1 ml) and PPD prepared separately from 3 atypical mycobacteria *M scrofulaceum M intracellulare* and *M avium* (atypical PPD at 0 1 μg/0 1 ml) were all obtained from Statens Seruminstitut Copenhagen for comparative skin testing One hundred μl of each was jected in the skin of the forearms after blood sam had been drawn for the lymphocyte experiments tests were read by one doctor after 48 and 72 h and judged as positive when the infiltrates (not erythema) measured at least 10×10 mm

PATIENTS

During a short time four children (2 boys and 2 g 18–34 months old were admitted to the Paediatric Cl in Malmo because of enlarged cervical lymph no (Table 1) In 3 children the left submandibular nodes v affected and in one the nodes along the left sternocle mastoid muscle In all the children the disease was lateral Routine laboratory tests showed normal hae globin concentrations and normal leukocyte counts I was increased in 2 patients slightly increased in one normal in one Ordinary bacteriological cultures of th specimens were negative in all the patients Exten serological tests for bacterial viral and protozoan in tions were negative

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Case report M M born August 28 1975 This p viously healthy girl was brought to our attention at age of 2 years and 10 months because of an enlarged submandibular lymph node (1×2 cm) ESR was sligh

[1] Performed by Dr Ingmar Juhlin Department of Clini Bacteriology Malmo

Table 2 *T lymphocytes and intracutaneous PPD tests*

SRBC Sheep erythrocytes

	SRBC binding lymphocytes		PPD skin tests			
Patient	Percentage	Per litre ×10 [9]	*M tuberculosis*	*M scrofulaceum*	*M intracellulare*	*M avium*
H H	67	2 5	Negative	Negative	Negative	Negative
J T	60	2 4	Negative	Positive	Positive	Positive
J A	53	2 6	Negative	Positive	Positive	Positive
M M	24*	0 61	Negative	Negative	Negative	Negative

* Lymphocytes from M M were tested for additional surface markers with the following results Receptors for Fc of IgG 21% receptors for complement 25% surface membrane IgM 12%

increased (14 mm) and the number of leukocytes was normal (5 6×10⁹/litre) as was her temperature Two weeks later the node was unchanged and a biopsy specimen was obtained by fine needle aspiration The histological examination showed granulomas epithelioid cells and multinuclear giant cells The lymph node was surgically removed Treatment with antituberculous drugs was given for 8 days in connection with the excision (Table 3) Atypical mycobacteria belonging to the *M avium intracellulare* complex were isolated from the excised lymph node The girl recovered and has now remained asymptomatic for 6 months The immunological analysis indicated however that this patient had a T lymphocyte deficiency (see Results)

RESULTS

Plasma immunoglobulin levels were normal in all 4 patients One girl (J A) occasionally had a high value of plasma IgM during the disease

The analysis of cell mediated immunity revealed a T lymphocyte deficiency in one of the patients (M M) who had only 24% SRBC-binding lymphocytes (Table 2) Seven weeks later the proportion of T lymphocytes was 14% which is also low (reference values, i e mean ± 2 S D of the laboratory 56–80%) The total numbers of SRBC binding lymphocytes were 0 6×10⁹/l and 1 2×10⁹/l respectively (Table 2) One patient (J A) had a borderline value of SRBC binding lymphocytes (53%) but the total number was normal The remaining 2 patients had normal proportions of SRBC binding lymphocytes

The lymphocytes from M M were tested for receptors for Fc of IgG and complement and for membrane bound IgM The results were normal

The lymphocytes of all the children were activated in a normal way when stimulated with the mitogen PHA

Skin tests with PPD from *M tuberculosis* were negative in all 4 children (Table 2) Skin tests with 3 different atypical PPDs were negative in M M and negative or uncertain in H H Two patients reacted more or less intensely to all PPDs except PPD from *M tuberculosis*

Lymphocytes were stimulated in vitro by a battery of different PPDs (Table 3) Control lymphocytes were obtained from BCG vaccinated individuals PPD from *M tuberculosis* was a significantly more efficient stimulator for control lymphocytes than any of the atypical PPDs Some of these, e g *M fortuitum* were virtually without effect In contrast, the patients' lymphocytes responded equally well or better to PPD from atypical mycobacteria than to PPD from *M tuberculosis* (J A and M M Nov 27 had low responses to *M fortuitum*) Thymidine incorporation measured in cpm, varied considerably between different individuals Of the patients J T had markedly high thymidine incorporation values also when stimulated with PPD from *M tuberculosis* Nevertheless, 3 of 4 atypical PPDs induced a still higher lymphocyte response in this patient (Table 3) At the first examination, M M had high stimulation ratios in tests with atypical PPDs, especially

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Purified tuberculin from *M tuberculosis*

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The affected lymph nodes were surgically removed In all 4 patients the histological examination of the lymph nodes showed changes typical of mycobacterial infection viz granulomatosis with epithelioid cells, giant cells and caseous necrosis Acid fast bacilli were not seen in the sections Atypical mycobacteria belonging to the *M avium intracellulare* complex were isolated from the *surgical specimens* in 2 patients (J T and M M)[1] In H H cultures for mycobacteria were negative and in J A no culture was performed None of the patients had had any known contact with tuberculosis infected persons Three of the patients were treated with antimycobacterial chemotherapy (Table 1)

Case report M M born August 28, 1975 This previously healthy girl was brought to our attention at the age of 2 years and 10 months because of an enlarged left submandibular lymph node (1×2 cm) ESR was *slightly*

[1] Performed by Dr Ingmar Juhlin, Department of Clinical Bacteriology, Malmo

among our patients M M had a positive culture of mycobacteria but no reaction in the skin tests This patient had a T cell deficiency which could explain the anergy Infections with atypical mycobacteria seem to be more common in the compromized host (20) including patients with a defect of cell mediated immunity and a negative skin test thus cannot exclude mycobacteriosis The interpretation of *skin tests* is further complicated by the variation in the response during the course of the disease (19)

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The concentration of PPD was 10 μg per ml

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H H	3 700	0 82	1 08	1 92	0 66
J T	18 300	1 54*	1 24	2 51**	1 84*
J A	5 600	0 85	1 26	1 02	0 31*
M M Oct 2	120	15 0	31 5**	11 4*	1 98
M M Nov 27	3 100	0 93	1 03	0 71	0 17*
Control 1	14 300	0 09**	0 04**	0 20*	0 03***
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m minus cpm with medium alone

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Asterisks represent level of significance (p below 0 05 0 01 and 0 001 respectively) when analysing difference tween mean [1] log cpm *M tuberculosis* and mean [1] log cpm atypical mycobacteria in Student s t test

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DISCUSSION

In countries where BCG vaccination of newborn infants is not the rule it has been possible to estimate the incidence of subclinical infection with atypical mycobacteria by skin testing Even though cross sensitization between different mycobacterial antigens frequently occurs, a strong skin reaction to atypical PPD together with a weak or absent reaction to PPD from *M tuberculosis* can be taken as evidence of an infection with an atypical mycobacterium (15 18, 21) In the United States the frequency of strong reactions to atypical PPDs in the population varies from 10–20% in some regions up to 70% in others, which far exceeds the frequency of strong reactions to ordinary PPD (7, 12) This shows that exposure and subclinical infectı with atypical mycobacteria is common Sti the incidence of clinical infection is low Me ingitis, pulmonary, skeletal and disseminat mycobacteriosis are very rare in children ar probably restricted to patients with diminishe resistance though only few cases have had thorough immunological work up (3, 16 20 Cutaneous mycobacteriosis, so called swim ming pool granuloma, may result from inocula tion of skin lesions with water containing *M marinum* (for review see 14) Clinical infec tions in small children mostly present as cer vical lymphadenitis, chiefly between the ages of 1 and 5 years (1 2, 6 8 14) Nothing in the histories of our patients suggested that the children had been exposed to an unduly large number of mycobacteria Accordingly, it seemed natural to look for factors of dimin ished host resistance in these patients

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[a] Net cpm=cpm with PPD in tissue culture medium minus cpm with medium alone
[b] Stimulation ratio Net cpm in lymphocyte cultures with atypical PPD divided by the corresponding value obtained with PPD from *M tuberculosis*
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Submitted May 21 1979
Accepted Dec 7 1979

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Acta Pædiatr Scand 59 517–523, 1980

THE EFFECT OF DIGESTIVE ENZYMES ON THE BINDING AND BACTERIOSTATIC PROPERTIES OF LACTOFERRIN AND VITAMIN B12 BINDER IN HUMAN MILK

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ABSTRACT Samson, R R , Mirtle, C and McClelland, D B L (University Department of Therapeutics and Clinical Pharmacology, The Royal Infirmary, Edinburgh, Scotland) The effect of digestive enzymes on the binding and bacteriostatic properties of lactoferrin and vitamin B12 binder in human milk Acta Paediatr Scand, 69 517, 1980.—Human milk contains unsaturated lactoferrin and vitamin B12 binding protein It has been suggested that these proteins may exert antibacterial effects in the intestine of the breast fed infant, but the effect of the intestinal environment on the antibacterial effect of these proteins has not been described In this study human milk was treated with pepsin and trypsin and the influence of digestion on iron and vitamin B12 binding capacity, bacterial uptake of iron and vitamin B12 [illegible] released iron from milk and abolished its bacteriostatic effect Trypsin digestion slightly reduced the molecular size of the vitamin B12 binding protein without releasing free vitamin B12 the bacteriostatic effect on a vitamin B12 dependant organism was, however, abolished In contrast, trypsin digestion did not affect iron binding or bacteriostatic effects attributable *to lactoferrin* The findings support an in vivo bacteriostatic role for lactoferrin in the breast fed neonate s intestine but do not support a similar role for the vitamin B12 binding protein

KEY WORDS Lactoferrin, vitamin B12 binding protein, human milk

In human colostrum and milk both iron and vitamin B12 are present and are bound to specific carrier proteins lactoferrin (11) and the vitamin B12 binding protein (7, 13) The biological importance of these binding proteins is unknown but it is well recognised that one potentially important property of lactoferrin is the bacteriostatic effect which it exerts by depriving certain microorganisms of iron (2, 3 4 10 11) By analogy it has been suggested that the highly unsaturated vitamin B12 binding protein might exert a bacteriostatic action by making vitamin B12 unavailable to bacteria which require it (5 8) It is attractive to speculate that these proven or postulated bacteriostatic mechanisms may help provide the breast fed infant with resistance to bacterial infection or may play a part in determining the distinctive intestinal microbial flora of the infant It is difficult to investigate these questions in vivo There is little information available from animal studies (4) and none from studies on human infants It is however possible to ask and answer some questions in vitro which may indicate whether these binding proteins could exert bacteriostatic effects in the infant s intestine

In this study we have subjected human milk to digestion with pepsin and with trypsin and investigated the effect of digestion on the ability of milk to bind iron and vitamin B12 We have attempted to answer the following questions

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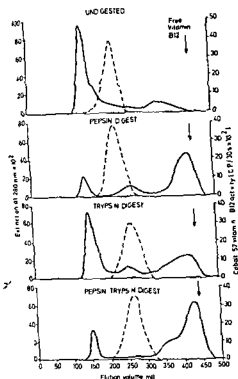
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1 The elution patterns obtained following Sephadex 00 gel filtration of cobalt 57 vitamin B12 labelled hu n milk after digestion with pepsin and trypsin — tinction at 280 nm cobalt 57 activity

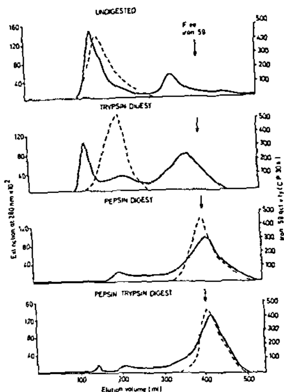


Fig 2 The elution patterns obtained following Sephadex G200 gel filtration of iron 59 labelled human milk after digestion with pepsin and trypsin —, Extinction at 280 nm, , *iron-59 activity*

ange in the protein content of the fractions early changes the elution position of the obalt-57 radioactivity suggesting a fall in olecular weight of the protein binding the belled vitamin B12 Sequential digestion with *epsin and trypsin produces changes in the* lution of both protein and radioactivity No ree vitamin B12 could be detected in any of hese experiments

To detect very small amounts of released vitamin B12, control and digested samples were dialysed No cobalt-57 labelled vitamin B12 *radioactivity was detected in the dialysis* fluids from control or pepsin digested samples After trypsin digested, 2 6% of the total cobalt-57 activity was dialysable, representing 130 pg of the 5 000 pg present in the sample

Fig 2 shows the results of similar experiments using milk labelled with iron-59 The results differ markedly *With pepsin digested* milk there is a marked shift in the elution position of iron-59 radioactivity indicating that all the iron has been released in the free state In the trypsin digested sample the iron-59 radioactivity eluted about 43 ml later than that in the control sample indicating a fall in molecular weight of the iron binding protein No free iron was liberated

Further studies of the effect of low pH on iron binding showed that after exposure to pH 2 for 1 hour in the absence of pepsin, 94% of the iron could be dialysed out of the milk showing that pepsin digestion was not required to release the bound iron Dialysis of the control and trypsin digested samples revealed no detectable release of iron-59

Bacterial uptake of cobalt 57 vitamin B12 and iron-59 from control and enzyme digested milk

E coli strains were unable to take up vitamin B12 from untreated control milk as previously

(i) Does enzyme digestion destroy the binding proteins or cause them to release iron or vitamin B12?

(ii) Can bacteria take up the iron and vitamin B12 in milk more easily after enzyme digestion?

(iii) Is the bacteriostatic effect of milk affected by enzyme digestion, and can this effect be attributed to changes in the binding of iron or vitamin B12?

Our findings provide support for an in vivo bacteriostatic function of lactoferrin but give no support to the view that the vitamin B12 binding protein may have bacteriostatic actions in the gastrointestinal tract

MATERIALS AND METHODS

Labelling of milk with iron and vitamin B12

A pool of normal human milk was labelled with sufficient cobalt 57 vitamin B12 to saturate 80% of the available binding capacity The methods of labelling and assessing binding capacity were described in detail by Samson & McClelland (13) A second aliquot of the pool was labelled with iron 59 One microcurie of iron 59 ferric citrate was added to 50 ml of milk and stirred for 1 hour at room temperature The added iron was sufficient to saturate 5% of the total lactoferrin After labelling the milk was stored in aliquots at −40°C

Enzyme digestion of cobalt 57 vitamin B12 and iron 59 labelled human milk

Aliquots of each pool were digested for 1 hour at 37°C with pepsin (Sigma 26C 8045) trypsin (Worthington TRSF 7AB) and with pepsin and trypsin sequentially using concentrations of 2 mg each enzyme per millilitre milk Pepsin digestion was carried out at pH 2 0 and trypsin digestion pH 8 0 The pH was adjusted with predetermined volumes of HCl and NaOH The pepsin digest was returned to pH 8 0 after 1 hour Samples of the labelled milk were incubated without enzymes as controls All samples were frozen at −40°C until further treatment

Chromatography of digested samples

After digestion 5 ml aliquots of each sample were passed through a 95 cm×2 5 cm column of Sephadex G200 at a flow rate of 10 ml per hour using 0 01 M tris HCl buffer pH 8 0 in 0 15 M Na Cl Each 5 ml fraction was monitored for extinction value at 280 nm Cobalt 57 or iron 59 activity was measured by scintillation counting The elution positions of free cobalt 57 labelled vitamin B12 and of free iron 59 were determined It was considered possible that during gel filtration of digested milk small amounts of free radioactivity liberated by digestion might not be detected as a result of dilution in the column An attempt was made to evaluate this by dialysing 1 ml aliquots of the digested milk and controls against 20 ml phosphate buffered saline pH 7 4 for 24 hours at 4°C after which the radioactivity of dialysates and dialysis fluids was determined

A further sample of the iron 59 labelled milk was incubated at 37°C for 1 hour at pH 2 0 and dialysed against N/100 HCl 2 litres at 4 C for 24 hours. The activity of the dialysate was then counted

Bacterial uptake of vitamin B12

Details of the method used have been described (14) Samples of undigested and digested milk labelled with cobalt 57 vitamin B12 were diluted 1 in 5 in a basal medium containing no vitamin B12 or iron Free cobalt 57 vitamin B12 was added to the medium alone to measure bacterial uptake of free vitamin Suspensions of bacteria harvested from slopes were adjusted to an extinction value of 0 4 at 450 nm in sterile phosphate buffered saline pH 7 4 One ml of suspension was added to each tube After incubation for 5 hours at 37°C the bacteria were centrifuged down (10000×g for 0 5 hour) and the radioactivity in the supernatant counted Several *E coli* strains were tested in this system (01 02 03 04 0? 014 055 and 0111 from the National Collection of Type Cultures and 8134 from the National Catalogue of Industrial Bacteria the latter is a vitamin B12 dependant strain)

Bacterial uptake of iron

Samples of undigested and digested milk labelled with iron 59 were treated in a similar manner as described above *E coli* 055 was added

Bacteriostatic effect of milk before and after digestion

Details of the methods have been described (14) Test incubation at 37°C by measuring the change in extinction of the culture and by counting colony forming units using a whole plate technique

RESULTS

Gel filtration of labelled milk before and after enzyme digestion

Fig 1 shows the protein content and cobalt 57 radioactivity of the fractions obtained by gel filtration of control and enzyme digested milk

Comparing the control sample with the pepsin treated milk there are two obvious changes The major protein peak in the control sample almost disappears with pepsin treatment but in contrast the peak of cobalt 57 radioactivity is unaffected However exposure to trypsin, although producing little

Table 2. *The extinction, viable count and uptake of cobalt-57 vitamin B12 by E. coli N C I B 8134 following incubation at 37°C/5 hours with undigested and digested pooled labelled human milk*

Nature of sample	Extinction 450 nm	Viable count ×10 •	Percent uptake of cobalt 57 vitamin B12
Undigested	0.15	17	5
Pepsin digest	0.12	9	4
Trypsin digest	0.94	790	87
Sequential digest	0.74	430	84

min B12 binding capacity of colostrum and milk is considerable (7, 13). The possibility that this binding capacity could influence bacterial ecology in the gastrointestinal tract has been suggested (5, 8). Although the vitamin is not taken up by a range of intestinal bacteria when in the bond state a bacteriostatic effect could only be demonstrated for a known vitamin B12 dependant *E. coli* (14). Many organisms which will grow in media lacking vitamin B12 will nevertheless take up the vitamin when it is present (5, 14).

In the present study we have examined the ability of the gastrointestinal enzymes pepsin and trypsin to release bound vitamin B12 from human milk and make it available to bacteria. Gel filtration of the labelled vitamin B12 binding protein (Fig. 1) indicates that the binding protein resists digestion with pepsin and that no dissociation of the bound vitamin occurs at pH 2.0. No free vitamin B12 was detected by gel filtration or dialysis. The activity of the pepsin used is indicated by the reduction in the initial protein peak and the appearance of smaller molecular weight material. Following trypsin digestion the vitamin B12 binding protein appears to be reduced in size. However, the area under the peak is very similar to that of the undigested control and there is no evidence that free vitamin is released, suggesting that any fragment(s) removed by digestion did not contain labelled vitamin B12. Ford (5) has shown that sow's milk also contains a vitamin B12 binding protein which is resistant to pepsin digestion and partly resistant to digestion with trypsin.

The uptake of the bound cobalt-57 vitamin B12 by *E. coli* (Fig. 3) shows the inability of these organisms to assimilate the bound vitamin. The action of pepsin, which produced no change in behaviour of bound vitamin B12 on gel filtration, is reflected in the inability of *E. coli* to take up vitamin B12 from pepsin treated milk. Digestion with trypsin not only decreased the apparent molecular size of the binding protein but permitted the test organisms to take up vitamin B12 from the milk. When fractions taken from the elution peaks of radioactivity were fed to the test organisms the uptake of vitamin B12 was very similar to that from the whole digest. It is not clear how the bacteria were able to remove vitamin B12 from the altered molecule since the vitamin was still firmly bound to a non dialysable moiety.

These results indicate that trypsin digestion of milk in the intestine will make the vitamin available for uptake by bacteria. It therefore seems unlikely that this protein could influence the intestinal flora by virtue of its vitamin B12 binding ability in the manner which has previously been suggested (5, 8).

Effect of digestion on iron-59 labelled pooled human milk

Unsaturated human milk lactoferrin inhibits the growth of a wide range of microorganisms and saturation with iron annuls this effect (2, 3, 4, 10, 11). If this bacteriostatic effect is to work in the neonatal gastrointestinal tract, lactoferrin must retain its iron binding capabil-

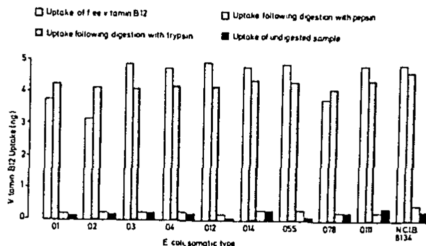


Fig. 3. The uptake of vitamin B12 from cobalt 57 vitamin B12 labelled human milk by 10 strains of *E. coli* following digestion of the milk with pepsin and trypsin.

described (13) or from pepsin treated milk. However, after trypsin digestion, the vitamin B12 was taken up by all the test organisms in similar amounts as the free vitamin (Fig. 3).

Iron uptake showed a different pattern (Table 1). Again little iron, only 3% of the added label, was taken up by bacteria growing *in the presence of untreated labelled milk*. Trypsin digestion did not affect bacterial iron uptake but pepsin plus trypsin digestion permitted bacteria to take up about 90% of the total iron-59 activity. Addition of purified unsaturated lactoferrin to a pepsin digest prevented bacterial uptake of iron, only 7% being taken up by the test organism.

Effect of enzyme digestion on bacteriostasis attributable to vitamin B12 and iron binding

We have previously shown that a bacteriostatic effect of vitamin B12 binding in milk can only be shown for vitamin B12 dependent organisms such as *E. coli* N.C.I.B. 8134 (14). Table 2 shows the effect of enzyme digestion on the ability of milk to influence growth of this organism. For these experiments excess iron was added to milk as previously described (14) to exclude bacteriostatic effect of lactoferrin. While growth was markedly inhibited in the presence of control milk or pepsin digested milk, digestion by trypsin or pepsin plus trypsin increased growth by approximately 1.5 log 10.

Using the non-vitamin B12 dependant E. coli 055, the effect of enzymes on the bacteriostatic effect of lactoferrin was investigated. Bacterial growth in the presence of pepsin digested milk was 1 log greater at 5 hours than in the presence of untreated or trypsin digested milk (Table 1).

DISCUSSION

Effects of digestion on cobalt-57 vitamin B12 labelled pooled human milk

Studies of the vitamin B12 content of human colostrum and milk have shown that the vitamin is presented to the infant in a protein bound form which is not readily assimilated by bacteria. Furthermore the unsaturated vita

Table 1. *The extinction, viable count and uptake of iron-59 by E. coli 055 following incubation at 37°C/5 hours with undigested and digested pooled labelled human milk*

Nature of sample	Extinction 450 nm	Viable count ×10⁻⁶	Percent uptake of iron 59
Undigested	0.15	102	3
Trypsin digest	0.38	131	11
Pepsin digest	0.88	1 020	87
Sequential digest	1.28	1 020	91

in human colostrum and milk Quantitation of the vitamin and its binder and the uptake of bound vitamin B12 in intestinal bacteria *Acta Paediatr Scand* 69 1979

4 Samson R R, Mirtle C & McClelland, D B L Secretory IgA does not enhance the bacteriostatic effects of iron binding or vitamin B12 binding proteins in human colostrum *Immunology* 38 367 1979

Submitted Sept 7, 1979
Accepted Dec 6 1979

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ity despite the attention of gastrointestinal enzymes

Examination of the gel-filtration elution profile or iron 59 labelled milk following pepsin digestion (Fig 2) shows that the initial protein peak has been almost totally digested Furthermore, iron dissociates from lactoferrin at pH 2 0 as shown in the present studies and by Groves (6) It therefore seems likely that exposure to the action of gastric acid and pepsin will annul bacteriostatic effects of lactoferrin which are due to iron deprivation Reassociation of iron with lactoferrin seems unlikely to occur at the higher pH values in the ileum as pepsin digestion of lactoferrin appears to be complete However, it cannot be assumed that milk proteins fed to an infant will be exposed to acid and pepsin at all stages of the feed Mason (12) showed in a study of 25 neonates between 5 and 13 days old that an initial pH value of 3 5 in the gastric contents rose following the ingestion of breast milk to pH 6 5 and fell slowly to pH 5 2 after 2 hours by which time most of the feed had left the stomach Henderson (9) in a radiological study showed that in neonates most of a feed passes directly into the duodenum Low concentrations of acid and pepsin following Histalog stimulation were found in neonates during the first four months of life (1) It is therefore probable that at certain stages of the feed conditions in the infants stomach may not permit the digestion of lactoferrin by acid and pepsin

Although digestion with trypsin reduced the apparent molecular size of lactoferrin there is no evidence in the present work that iron was liberated Table 1 however shows that slightly more growth of the test organism took place following trypsin digestion and this is reflected by a small increase in the uptake of radioactive iron Nevertheless, if the acid and peptic activity of the neonatal stomach are insufficient to destroy lactoferrin it is likely that the protein could exhibit a bacteriostatic effect in the small intestine despite the presence of trypsin

The present findings support the possible bacteriostatic role of lactoferrin in the intestine of the breast fed infant but offer no support for the suggestion that the vitamin B12 binding protein in milk could influence bacterial growth in the intestine The data, however, emphasise the need for better information about conditions in the stomach and upper small bowel of the normal infant before the host protective function of proteins in breast milk can be adequately understood

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Acta Pædiatr Scand 69 525–529, 1980

INFANT FEEDING PRACTICES AMONG NURSING PERSONNEL IN MALAYSIA

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ABSTRACT. Sinniah, D , Chong, F. M. and Aroklasamy, J. (Departments of Paediatrics and Social and Preventive Medicine, University of Malaya and the School of Nursing, Univer-

infant feeding practices among nursing personnel from representative centres using questionnaires. It was found that although 75% of mothers breast fed their babies at birth only 19% did so at 2 months and 5% at 6 months respectively. Chinese mothers initiated breast feeding less frequently compared with Indian or Malay mothers. The prevalence of breast feeding was higher among lower category nurses, lower income groups and those from health centres. Decision for breast feeding was based in most instances on conviction derived from reading, lectures or advice from relatives. The vast majority of mothers listed 'work' as the main reason for termination of breast feeding followed by 'insufficient breast milk' and satisfactory past experience with bottle feeding The ramifications of these findings and measures to improve the prevalence and duration of breast feeding are discussed

KEY WORDS Infant feeding, breast feeding, nursing personnel

There is a significant decline in the incidence and duration of breast feeding in many industrialised nations, and more recently corresponding trends have been observed in developing countries (4) These changes have serious implications for the nutritional welfare of young infants as the remarkable ability of malnourished women to breast feed their babies for prolonged periods is the most redeeming feature in an otherwise bleak nutritional situation in many countries (3)

The prevalence of breast feeding has fallen in both the urban and rural areas of Malaysia (2 6) This alarming decline in breast feeding has stimulated the need for a critical review of infant feeding practices among one important group of subjects—the nursing personnel who come in close contact with mothers and play a significant role in influencing the community through health education, dietary advice and campaigns to promote breast feeding

MATERIALS AND METHODS

A survey was carried out among nursing personnel using questionnaires The questions were formulated to obtain accurate and pertinent information relating to breast feeding and artificial feeding practices without disclosure of the identify of the respondent

Nursing staff of all categories, from matrons to midwives in 5 representative centres in both urban* and rural areas were selected The centres were 1 University Hospital, Kuala Lumpur (UH, K L)* 2 General Hospital, Kuala Lumpur (GH K L)* 3 General Hospital, Kota Baru (GH K B)* 4 District Hospital Kajang (DH K)

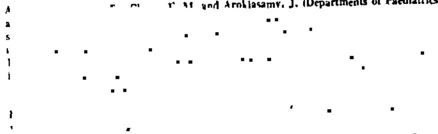
Hospitals and Rural Health Centres

Epidemiological data on infant feeding practices by nursing staff were compiled and analysed according to (a) age group (b) race (c) designation (d) income and (e) centre

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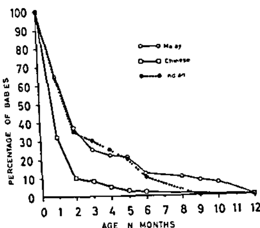


Fig 2 Duration of Breast feeding by race

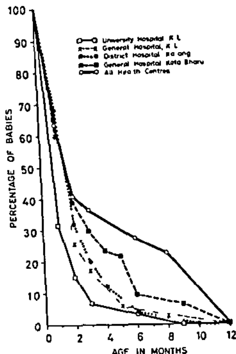


Fig 4 Duration of breast feeding by hospital centres

come and was found to be significantly higher in the lower income groups ($p<0.01$) The median duration of breast feeding in families with a monthly income of <US$ 500 was 2 7 months compared with 2 0 and 1 8 months in those with an income of US$ 500–850 and US$ 850–1100 respectively

Centre The relation between the centre where the mother worked and the prevalence and duration of breast feeding is seen in Fig 4 There is a definite relationship between the centre and the infant feeding pattern The prevalence of breast feeding was highest among mothers in the Health Centres followed by General Hospital in the East Coast (GH K B) District Hospital in the West Coast (DH K) General Hospital Kuala Lumpur and University Hospital respectively The differences were statistically significant ($p<0.001$) The median duration of breast feeding was shortest amongst the University Hospital staff (1 7 months) followed by the staff of the General Hospital Kuala Lumpur (2 3 months) The median duration of breast feeding with other centres ranged from 2 5 to 2 6 months

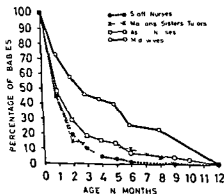


Fig 3 Duration of breast feeding by designation of mother

Table 2 *Reasons given for breast feeding by 251 mothers*

Reasons	No of mothers	% of mothers
Own conviction	156	62 2
Lectures	88	35 1
Reading	87	34 7
Advice from mother/relative	49	19 5
Past experience	49	19 5
Other reasons	11	4 4
Total number of reasons	440	

Table I *Nursing population marital status, number of questionnaires sent and response received from the various centres*

	UH K L	GH K L	GH K B	DH K	RHC U L
Total nursing population	745	1230	501	106	25
Total no of married nurses	384	962	326	98	23
Total no questionnaires sent	130	100	100	80	23
Total no of responses	100	64	74	55	23
Percentage of number of responses	77	64	74	69	100

In addition the reasons for breast feeding and for pre term termination of breast feeding were computed

RESULTS AND DISCUSSION

The nursing population, their marital status, the number of questionnaires sent and the number of responses received are recorded in Table I

A total of 317 (72%) positive responses were received to 433 questionnaires sent to the 5 selected centres

It was observed that 237 (74 8%) of the 317 mothers elected to wholly or partially breast feed their babies at birth But the figure fell

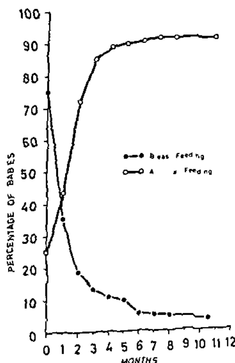


Fig 1 Infant feeding practices among nursing personnel

to 35 5% at 1 month, 19% at 2 months, 13 5% at 3 months, 5 4% at 6 months and 3 5% at 9–12 months as seen in Fig 1

Factors influencing breast feeding

Age The prevalence of breast feeding was compared between nurses of different age groups but no significant differences were observed ($p>0\ 5$) Older mothers (41–50 years) appear to breast feed their babies longer than young mothers who generally stopped breast feeding by 6–9 months

Race The frequency and median duration of breast feeding were compared between the races (Fig 2) Chinese mothers were found to breast feed their babies less frequency (53 5%) than Indian (80%) or Malay mothers (90 79) These differences were statistically significant ($p<0\ 001$) The median duration of breast feeding by Chinese mothers was 1 74 months compared with 2 5 and 2 53 months by Indian and Malay mothers respectively

Staff designation of mother The prevalence and duration of breast feeding were compared between mothers of different designations (Fig 3) The lowest designation mother, namely the midwife was found to breast feed more often compared with higher designation mothers This difference is statistically significant ($p<0\ 001$) The median duration of breast feeding by matrons sisters and tutors was 1 9 months compared with 1 92 2 3 and 3 75 months by staff nurses assistant nurses and midwives respectively

Family income The frequency of breast feeding was studied in relation to family in-

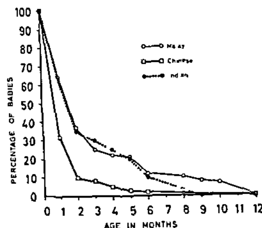


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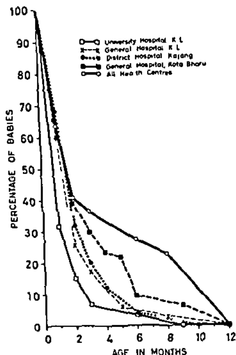


Fig 4 Duration of breast feeding by hospital centres

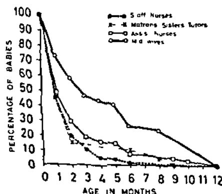


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Table 3 *Reasons for pre-term termination of breast feeding by 305 mothers*

Reason	No of mothers	% of mothers
Work	261	85.6
Insufficient breast milk	118	38.7
Past experience	33	10.8
Do not want to be tied down to baby	18	5.9
Advise from mothercraft nurse	13	4.3
Advise from mother	7	2.3
Advise from relative	3	1.0
Everybody doing so	3	1.0
Other reasons	25	8.2
Total number of reasons	483	

Reasons for breast feeding

The reason given by mothers for electing breast feeding are summarised in Table 2. A total of 440 reasons were given by 251 mothers of whom 62.2% listed their own conviction as the main reason for breast feeding followed by reading 34.7%, lectures 35.1%, advice from mother or relatives 19.5% and past experience 19.5% respectively.

Reasons for termination of breast feeding

The reasons for not initiating or for pre-term termination of breast feeding in favour of formulae feeding are listed in Table 3.

305 mothers gave 483 reasons. 12 did not complete the questionnaires. The main reasons were work (85.6%) and insufficient breast milk (38.7%) followed by satisfactory previous experience with bottle feeding (10.8%) and reluctance to be tied down to the baby (5.9%). Most mothers discontinued breast feeding once they resumed work, generally six weeks after delivery. They found it difficult to combine full time work and breast feeding especially when they were away from home for long periods.

Despite the steady decline in breast feeding in both the urban and rural areas of Malaysia (1, 2), 75% of the nursing staff from representative medical centres in Malaysia were motivated adequately enough to initiate breast feeding. This frequency is similar to the incidence of breast feeding during the first week in rural areas (77.5%) and far higher than the national average (64%) and that in the urban areas (47%) of Malaysia (6). The motivation was high among our nurses because of conviction resulting from their education, training, experience and advice they received. However by 3 months only a mere 13.3% were breast feeding as compared with the national average of 21–32% (6). The frequency at 6 months (5.4%) is easily one of the worst on record (5). Chinese mothers had the lowest breast feeding rates compared to Malays and Indians suggesting differing cultural influences in the community.

The alarming decline in the frequency and duration of breast feeding by nursing staff who should be setting an example for the people of Malaysia has been scrutinised in our study. The main reasons for premature termination of breast feeding were work and insufficient breast milk and these are probably representative of the problems faced by most working mothers. The syndrome of partial breast feeding with supplementary feeding, inadequate sucking on the breast followed by decreased prolactin reflex and lactation failure is well known. In addition the period of maternity leave in Malaysia is only 42 days compared to 7 months in the Nordic countries, and our nurses are further handicapped by lack of facilities to encourage breast feeding at their place of work, so much so that we are now faced with the paradox where developing countries like Malaysia have to catch up with the upward trend in breast feeding in developed countries like Sweden (7).

It is obvious that not only is strong motivation a pre-requisite to successful breast feeding but facilities should be available for working mothers to breast feed their babies in creches attached to their place of work. There is also urgent need for legislation to extend the period of maternity leave.

Concerned by the disturbing results of this study, a breast feeding promotion group has

been formed at the University Hospital to encourage breast feeding by the nursing staff

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Submitted Aug 27 1979
Accepted Nov 29 1979

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Reasons for termination of breast feeding

The reasons for not initiating or for pre term termination of breast feeding in favour of formulae feeding are listed in Table 3

305 mothers gave 483 reasons, 12 did not complete the questionnaires The main reasons were work (85 6%) and insufficient breast milk (38 7%) followed by satisfactory previous experience with bottle feeding (10 8%) and reluctance to be tied down to the baby (5 9%) Most mothers discontinued breast feeding once they resumed work generally six weeks after delivery They found it difficult to combine full time work and breast feeding especially when they were away from home for long periods

Despite the steady decline in breast feeding in both the urban and rural areas of Malaysia (1, 2) 75% of the nursing staff from representative medical centres in Malaysia were motivated adequately enough to initiate breast feeding This frequency is similar to the incidence of breast feeding during the first week in rural areas (77 5%) and far higher than the national average (64%) and that in the urban areas (47%) of Malaysia (6) The motivation was high among our nurses because of conviction resulting from their education, training, experience and advice they received However by 3 months only a mere 13 3% were breast feeding as compared with the national average of 21–32% (6) The frequency at 6 months (5 4%) is easily one of the worst on record (5) Chinese mothers had the lowest breast feeding rates compared to Malays and Indians suggesting differing cultural influences in the community

The alarming decline in the frequency and duration of breast feeding by nursing staff who should be setting an example for the people of Malaysia has been scrutinised in our study The main reasons for premature termination of breast feeding were work and insufficient breast milk and these are probably representative of the problems faced by most working mothers The syndrome of partial breast feeding with supplementary feeding inadequate sucking on the breast followed by decreased prolactin reflex and lactation failure is well known In addition the period of maternity leave in Malaysia is only 42 days compared to 7 months in the Nordic countries, and our nurses are further handicapped by lack of facilities to encourage breast feeding at their place of work so much so that we are now faced with the paradox where developing countries like Malaysia have to catch up with the upward trend in breast feeding in developed countries like Sweden (7)

It is obvious that not only is strong motivation a pre requisite to successful breast feeding but facilities should be available for working mothers to breast feed their babies in creches attached to their place of work There is also urgent need for legislation to extend the period of maternity leave

Concerned by the disturbing results of this study, a breast feeding promotion group has

Acta Pædiatr Scand 69 531–532, 1980

LETTER TO THE EDITOR

VALIDITY OF URINALYSIS IN DIABETIC CHILDREN

Sir

I agree with Ludvigsson & Svensson about patient control of juvenile diabetes with urinalysis (6) These authors conclude that the majority of patients easily accept the method and that it is a valuable tool in the management of diabetes I would like to comment on two points 1) The usefulness of urinalysis in the management of the diabetic child Differences in opinion about the measurement of glycosuria in the treatment of juvenile diabetes still exist Malone et al (7) expressed doubts about the role of urine measurements after comparing urinary glucose concentrations in first and second voided specimens with plasma glucose levels They reported a group of children in whom mean plasma glucose levels was 233±51 mg/dl in the presence of negative urine tests In this case changing insulin dosage according to urinary glucose concentration would be of little value We determined blood glucose levels in diabetic children with negative glycosuria (3) Glucose concentrations associated with negative urine sugar were always under 210 mg/dl Although the amount of sugar in the urine gives only a rough indication of fluctuations in blood glucose between micturition it is a simple way of monitoring diabetic control We recommend urinalysis 4 times daily with the 2 drop Clinitest that permits estimation of glycosuria from 0 25% to 50% (1 2) The clinical value of the 2 drop Clinitest has been recently confirmed by other pediatric groups (5 8) 2) The attitude towards urinalysis We too felt at first that only a few children find urine tests a psychological problem but we have observations about the validity of information gathered in daily records Ernould & Graff have shown that 13 of 60 young diabetics knowingly falsified their records (4) They propose to send a specialised social nurse to the home to confirm or to disprove possible cheating

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The Editor asked doctor Ludvigsson to co ment on doctor Dorchy s letter

Sir

It is our experience that most children a adolescents do have a renal threshold for g cose below 10–11 mmol/l (180–198 mg/c

SHORT COMMUNICATION

MATERNO FETAL TRANSMISSION OF HEPATITIS A ANTIBODY

The prevalence of hepatitis A antibody (anti HAV) varies among different parts of the world In the newborn it is supposed that the percentage of positive results for anti HAV may reflect that of women of childbearing age and is due to passive transfer of immunity across the placental barrier falling as soon as the maternal immunoglobulin disappears In this study we have investigated the presence of hepatitis A antibody in a group of pregnant women and in children less than ten years old

Fifty pregnant women aged between 17–38 years (mean 25 years) were studied Blood was collected immediately before delivery from the antecubital vein and just after delivery from the umbilical cord In addition 209 children older than 4 weeks and younger than 10 years were investigated the ages of these children are shown in the table Hepatitis A antibody was determined by Radioimmunoassay (HAVAB Abbott) The results are shown in the table

Our findings confirm materno fetal transmission of anti HAV After three months anti HAV begins to disappear and between 9 months and 3 years the percentage of children with anti HAV is very low After this age positive results increase As we have previously shown (6) and as is also seen in the women in this study between the ages of 10 and 30 years most people in our area become anti HAV positive

In New York Cherubin and coworkers found no positive results in children less than 10 years old (1) and Stevens et al found 8 8% positive for anti HAV at the same age in different parts of the USA (5) Eighteen percent of teenagers are positive according to Tabor et al (5) and among children under 15 years Maynard et al found 2% positive (3) In Germany Frosner et al (2) have shown that most of the population becomes positive between 20 and 29 years of age possibly when they travel to Southern Europe These differences may depend on lower hygienic conditions which may favour the spread of hepatitis A virus at an early age, soon after passively transferred antibody has been lost

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Table 1 *Results of anti HAV screening*

Age	Number	Anti HAV positive	% positives
Mothers	50	49	98
Newborn	50	49	98
4–12 weeks	23	21	91
3–6 months	22	11	50
6–9 months	17	7	44
9–12 months	8	–	0
1–3 years	61	11	18
3–10 years	78	23	29

Thus a single-voided urine without glucose shows that the blood glucose level has fluctuated below 10 mmol/l. In managing insulin dependent diabetic children this may be more valuable than the snap-shots obtained by measuring blood glucose values, although we believe that self-determination of blood glucose at home may be useful in some patients When the methodological problems have been solved and we know more about the kinetics of Haemoglobin A1c, this may become another valuable parameter in the definition of control, especially in cases where Haemoglobin A1c values are normal or near normal Haemoglobin A1c will, however, never become a tool in the daily management of diabetes as it gives no information about what has been wrong As to attitudes towards urinalysis at home, we know that urinalysis and many other rules recommended by the adults are opposed at times, especially in the adolescent Whether patients falsify or not depends to great extent on their contact with adults, including doctors If patients expect criticism when they admit that they did not want to test their urine or when the degree of control is inadequate, they sometimes prefer to write down false values However, they may be inclined not to do so if they know and understand why they test their urine, how they can draw conclusions from the results and know that they can discuss problems Perhaps we should talk less about "cheating" and ask ourselves how we can improve psychological support for our brave patients

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SHORT COMMUNICATION

GASTRIC FINDINGS IN ADOLESCENTS TREATED FOR GRAVES DISEASE

It is well known that Graves' disease is often associated with other autoimmune diseases In the adult population this association most frequently involves atrophic gastritis (2 7) Furthermore Irvine et al (3) observed that approximately 3% of patients with autoimmune achlorhydric gastritis per follow-up year progress to latent pernicious anaemia

Abnormal gastric mucosa has also been revealed in autoimmune thyroiditis and hypo

Graves disease We therefore considered gastric investigations important for juvenile patients with Graves disease especially with a view to their long term prospects

During the period 1965–1976 we treated 40 patients for hyperthyroidism Criteria for the diagnosis and the clinical data have been presented elsewhere (6) After a follow up period of 0 5 to 10 2 (mean 4 4) years 33 patients (29 females 4 males) underwent gastric investigations The patients ages ranged from 11 1 to 24 8 (mean 19 1) at the time of the study

Methods for taking gastric biopsies classifying the gastric histology determining the serum vitamin B_{12} level and antibodies against gastric parietal cells nuclei intrinsic factor (5) thyroglobulin and thyroidal microsomes (6) have been reported earlier The gastric acid secretion was studied with the pentagastrin test and the reference values described by Dodge were used (1)

The gastric mucosa was abnormal in seven (26%) of 27 patients studied (Table 1) The mucosal damage was slight or moderate, whereas the most severe form, gastric atrophy was not found at all Achlorhydria was merely found in three and a subnormal response to pentagastrin stimulation (hypochlorhydria) in four out of 29 patients studied One of the two patients with atrophic gastritis had achlorhydria and the other had hypochlorhydria Slight mucosal changes associated with hypochlorhydria in two and a normal mucosa was found in two achlorhydric and in one hypochlorhydric patient Twelve (36%) had gastric parietal cell antibodies, but only one of them had achlorhydria and two had hypochlorhydria The serum B_{12} level in each patient was normal None had antibodies against intrinsic factor or nuclei Thyroglobulin antibodies were found in titres of ≥1 25 in 12 and microsomal antibodies in titres ≥1 10³ in 23 of 27 subjects who underwent gastric biopsy

According to Siurala et al (8) the frequency of gastritis is 29% in the age group 16–30 years in a Finnish rural community In their study the degree of gastritis was mainly mild only four of the 48 subjects (8%) had atrophic gastritis Gastric parietal cell and intrinsic factor antibodies were discovered in the age group 16–50 years in 31% and 1%, respectively (4)

The prevalence or the degree of gastritis in our patients did not differ from those in a normal population (8) Our patients with autoimmune thyroiditis or hypothyroidism (5) had more (39%) and severer forms of gastritis compared with the present series but the difference is not statistically significant Gastric parietal cell antibodies were, however, significantly more often found (in 36%) than in normal population (4) (χ^2 27 7, $p<0\ 001$), but

Acta Pædiatr Scand 69 537–545, 1980

CASE REPORT

PANCREATIC B CELL FUNCTION AND ABNORMAL URINARY PEPTIDES IN A BOY WITH LIPOATROPHIC DIABETES AND STENOSIS OF THE AQUEDUCT OF SYLVIUS

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ABSTRACT Hager, A Heding, L G, Larsson, Y, Ludvigsson, J and Trygstad, O (Department of Paediatrics, University Hospital, Linkoping, Sweden, Novo Research Institute Bagsvaerd, Denmark and Department of Paediatrics, Rikshopitalet, Oslo, Norway) Pancreatic B-cell function and abnormal urinary peptides in a boy with lipoatrophic diabetes and stenosis of the aqueduct of *Sylvius* Acta Paediatr Scand, 69 537, 1980 A boy with the classical clinical manifestations of acquired lipoatrophic diabetes has been studied for 5 years from the onset of diabetes at age 13 At the age of 15 a ventriculo-cisternal shunt operation was performed because of stenosis of the aqueduct of Sylvius, followed by a dramatic improvement in his diabetic state with a decrease of the 24 hr insulin requirement from 130 to 32 units After 12 months there was a relapse with increased insulin requirement up to the preoperative level Pimozide treatment was given for 7 months with no effect on the metabolic derangements Extremely high basal levels of serum C-peptide and pro-insulin were found throughout the period of observation A further increase occurred after i v arginine infusion tests indicating hyperfunctioning B-cells Repeated screenings of peptides in the urine by sephadex chromatography revealed pathological patterns similar to those observed in patients with other hypothalamic disorders, but different from that found in the urine of patients with congenital generalized lipodystrophy Injection into mice of peptides extracted from the preoperative urine produced an acute hyperglycemia The mechanisms behind this hypothalamic syndrome are unknown, but it is postulated that the abnormal urinary polypeptides originate from disorganized hypothalamic centres and that these peptides may be responsible for the disturbed carbohydrate and lipid metabolism.

KEY WORDS C peptide lipoatrophic diabetes, urinary polypeptides

Lipoatrophic diabetes is a rare and enigmatic disease first described by Lawrence in 1946 (18) The condition exists in both the congenital and acquired forms furthermore, the atrophy may be total or regional Classical manifestations are insulin resistant non ketotic diabetes hypertriglyceridemia, hepatomegaly due to fatty infiltration paucity or absence of subcutaneous and visceral fat depots, enlarged muscles and skin pigmentations (acanthosis nigricans) The prognosis of the disease is poor due to diabetic angiopathy and liver disease cirrhosis, hepatic failure and gastric haemorrhage (1, 24, 27) The condition is associated with hypothalamic dysfunction It is thought that both abnormal humoral and neural stimuli may induce insulin resistance and loss of fat depots (1, 6, 7, 20, 21, 22, 24, 26, 29)

We report here a case of the acquired type, which has been monitored for 5 years with repeated tests of B-cell function, and chromatographic analyses of urinary peptide patterns

Table 1 *Relation between gastric parietal cell antibody titres and gastric histology*

Parietal cell antibody titre	Normal gastric mucosa	Slight gastritis	*Atrophic* gastritis	No biopsy	Total number of patients
0	14	3	1	3	21
1 10	1			2	3
1 50	3				3
1 100	1	1			2
1 1000	1				1
1 10000				1	1
1 50000			1		1
1 500000		1			1
Total	20	5	2	6	33

there was no correlation between the presence of gastric parietal cell antibodies and the gastric histology or acid secretion This may be explained in many ways, e g the presence of gastric parietal cell antibodies might be only an immunological phenomenon without clinical significance Rather our findings may be explained by the younger age of our patients, closer correlation of the presence of antibodies and the clinical disease being found in older age groups as in autoimmune thyroiditis This presence of gastric parietal cell antibodies may anticipate gastritis Although parietal cell antibodies and gastritis are common in young patients with Graves disease the pernicious anaemia is to be found in older age groups

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Table 1 *Blood glucose, insulin antibodies (expressed as IgG), human C-peptide (normal fasting level <0 70) pancreatic glucagon (normal fasting level 0 11–0 24) and human proinsulin (normal fasting level <0 01) in different types of loading test*

Type of test	Duration of diabetes (years)	Time (min)	Blood glucose (mmol/l)	IgG (mU/ml)	Human C peptide (pmol/ml)	Pancreatic glucagon (ng/ml)	Human proinsulin (pmol/ml)
IVGTT	1 9/12	0	8 7	0 235	3 15	–	0 60
		10	14 9		3 90		0 75
		20	13 1		3 75		1 05
		30	11 3		3 90		0 75
		40	10 6		3 75		0 60
		50	9 7		4 20		0 75
		60	10 2		3 90		0 60
		90	7 5		3 45		0 60
IVGTT	2 7/12	0	9 0	–	1 02	–	0 74
		10	15 2		0 85		0 48
		20	14 5		1 02		0 80
		30	13 5		1 02		0 72
		40	12 9		1 02		0 68
		50	12 3		1 19		0 64
		60	12 1		1 02		0 69
		90	10 3		1 19		0 80
I v arginine	2 7/12	0	10 2	–	1 19	0 19	0 84
		10			2 38	0 32	1 09
		20	–		2 38	0 44	1 34
		30	13 6		2 55	0 47	1 38
		60	13 2		1 87	0 23	1 34
		90	–		1 53	0 19	1 06
I v arginine	4	0	7 1	0 245	0 68	0 14	0 45
		10	7 6		1 08	0 22	0 50
		20	7 8		1 10	0 22	0 57
		30	7 0		1 25	0 37	0 75
		40	7 7		1 38	0 26	0 75
		50	7 9		1 20	0 25	0 52
		60	7 5		1 15	0 18	0 57
		90	7 0		0 88	0 13	0 80
IVGTT	4	0	7 4	0 267	0 68	0 13	0 50
		5	14 0		0 55	0 12	0 40
		10	17 4		0 53	0 14	0 42
		20	16 0		0 65	0 12	0 42
		30	15 5		0 93	0 14	0 47
		40	17 0		0 98	0 12	0 50
		50	13 2		1 08	0 12	0 55
		60	13 0		1 05	0 12	0 47
		90	12 8		1 20	0 12	0 47
		120	9 1		1 18	0 14	0 55
Standardized breakfast	5 2/12	0	17 8	0 179	0 68	–	0 30
		30	17 9		0 98		0 40
		60	21 2		1 20		0 40
		90	21 2		1 13		0 50
		120	22 1		1 28		0 55

... were normal and there was no proteinuria on any occasion

From the onset of diabetes the patient has been on insulin treatment except for a period of 3 months in 1974 when there was a complete clinical remission From the end of 1974 he has been treated with insulin (monotard and Actrapid (Novo) in the morning and ... Monotard ... in Fig 2, ... based on ... Clinitest ... ays after ... mporary ... a reduc

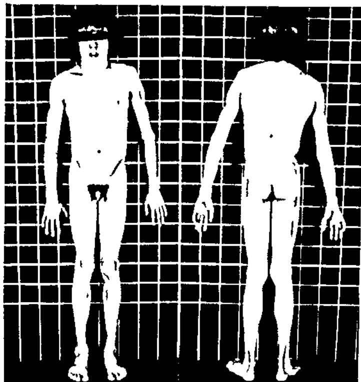


Fig 1 The patient at age 14

CASE REPORT

The patient, a boy, was born prematurely in 1959 with a birth weight of 2440 g The neonatal period was completely normal There was no consanguinity, he had 2 healthy siblings, and diabetes mellitus was unknown in the family During the first years of life the boy had frequent upper respiratory tract infections often associated with massive enlargement of the cervical lymph nodes Therefore adenoidectomy was performed A bone cyst of the left humerus caused a pathological fracture at age 10 In April 1973, at age 14, mild diabetes without ketoacidosis was diagnosed and insulin treatment was started At this time he was admitted to the Department of Paediatrics, University Hospital, Linkoping

On admission a near total lipoatrophy was noted (Fig 1), in accordance with the mother s observation of a gradual loss of subcutaneous fat since 7 years of age The paucity of subcutaneous fat was verified by skinfold measurements and soft tissue radiographs In addition the boy exhibited a protuberant abdomen with slight hepatic enlargement, enlarged muscles phlebomegaly abundant curly hair, and acanthosis nigricans in the axillary regions verified by a skin biopsy Height weight, skeletal age (according to Greulich & Pyle) and pubertal signs corresponded to chronological age X rays of all long bones revealed a bone cyst in the left tibia A radiogram of the skull showed signs of increased intracranial pressure which gave no symtoms Pneumoencephalography (and a subsequent ventriculography) revealed grossly enlarged ventricles, expecially the 3rd ventricle and a total stenosis of the Sylvian aqueduct A ventriculo-cisternal shunt operation was performed at age 16 However subsequent

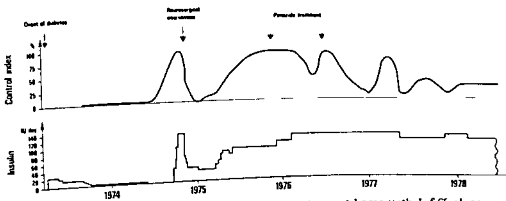


Fig 2 The metabolic control and insulin dose during the observation period Control index percent of urinalyses at home with 1–5% glucosuria A low control index thus means a good metabolic control

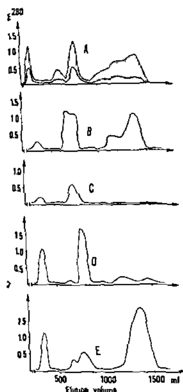


Fig 4 Sephadex G-25 gel chromatography of precipitates from urine samples obtained from the patient (A) Absorbancy pattern for normal control urines (±2 S D) (B) before shunt operation (C D E) immediately after 12 and 29 months after the shunt operation

All the Sephadex G 25 gel filtrations of the urine precipitates showed different patterns, and except for the specimen obtained 12 months after the shunt operation, they were all significantly different from those of normal control urine precipitates (Fig 4)

Of the crude urine precipitates a total dose of 8 mg per animal was injected for 20 days, subcutaneously into 10 albino mice No changes occurred in consumption of feed or oxygen, body weight or blood glucose However, another short-term experiment with injection into 5 mice of total dose of 4 mg per animal of the preoperative precipitate gave a significant increase in the blood glucose level as compared with control urine precipitate (Table 2)

DISCUSSION

Our patient demonstrated the classical manifestations observed in lipoatrophic diabetes In addition the abundant and curly hair is typical for the disease (24) as well as the bone cysts It has been postulated that paucity of fat in the bone marrow might produce cyst formation (8, 9)

Lipoatrophic diabetes seems to be induced by severe infections (1, 24), and the onset of the disease in our patient may have been precipitated by his recurrent respiratory infections These may have induced a slowly developing 'gliosis' in the brain tissue around the aqueduct of Sylvius (2), eventually causing a total aqueduct stenosis, and subsequently hypothalamic derangements A hypothalamic disturbance is considered pathogenetic in lipoatrophic diabetes, in both the congenital and acquired forms (1, 6, 22, 24, 26) Via abnormal neurohumoral signals a loss of fat in the subcutis and internal depots is induced concomitant with insulin resistance (1, 21, 24, 29) How these signals act in detail is not known, but in both animal experiments and clinical studies, urinary peptides have been found with adipo-

Table 2 *Blood glucose in mice (n=5) injected with 4 mg of peptides isolated from the patient s urine before shunt operation (I) and from control urine (II)*
Values are means and S D

	Time (hours)			
	0	1	4	7
Blood glucose mmol/l				
I	5 7±0 4	8 8±0 7	7 4±0 6	6 1±0 5
II	5 9±0 3	5 6±0 4	5 6±0 3	5 8±0 5

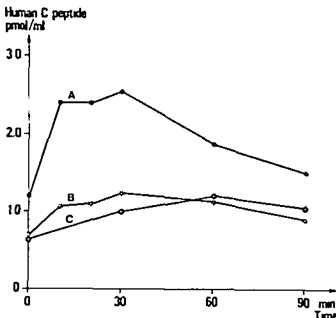


Fig 3 C peptide levels during different types of test (A) *I v arginine test 31 months after onset of diabetes* (B) I v arginine test 48 months after onset of diabetes (C) Standardized breakfast 62 months after onset of diabetes

tion in the insulin dose from 136 to 32 units per day Despite continuous glucosuria and blood glucose values between 30 and 35 mmol/l in the years 1975–77 ketonuria never occurred He was recommended to follow a diabetic diet of 2400 kcal/day (10 MJ) but he had great difficulty in complying with this recommendation Pimozide (a dopaminergic blocking agent) was given for 7 months in a dose of 6–10 mg/day (Fig 2) Side effects (drowsiness and fatigue) were noted but there was no improvement of the diabetic control and no return of subcutaneous fat

METHODS

Serum C peptide and proinsulin determined according to Heding (12 13) were followed in connection with the following tests 1) Intravenous glucose tolerance test (IVGTT) with 0 5 g glucose/kg body weight 2) Intravenous arginine infusion (*0 5 g/kg body weight for 30 minutes*) 3) Before and after a standardized breakfast containing 20% of the total daily energy intake with 45% as carbohydrates 35% as fat and 20% as protein

Plasma glucagon was determined using anti serum K964 specific for the C terminal part of the glucagon molecule and having a reactivity of gut glucagon like immuno reactivity less than 0 2% (11 14)

Insulin antibodies were measured as described by Christiansen (4)

Blood glucose was assayed according to the hexokinase method of Wright et al (35)

... cholesterol was determined

... Blood levels of T_4 T_3 TSH ... and LH were analyzed with routine mehtods

Urinary peptide patterns Urine from the patient was collected in 24 h specimens before shunt operation immediately afterwards and 12 and 29 months later Protein present in the specimens was precipitated according to a method described previously (7) The urinary peptides were fractionated on a Sephadex G 25 gel column (3×143 cm) by dissolving the precipitate in 30 ml 0 1 M ammo-

read at 260 nm The patterns of chromatograms have been reproducible and consistent for normal controls as well as for metabolic and behavioral disorders However the patterns might change on medical treatment (34)

RESULTS

Repeated fasting blood analyses showed serum triglyceride levels from 2 2–10 6 mmol/l (normal upper limit 1 8), cholesterol 5 1–7 2 mmol/l (<6 5), ALAT 0 51–1 68 μkat/l (<0 80) and 0 61–2 10 μkat/l (<0 70) The triglyceride values were highest in periods of poor diabetic control

Examinations of the release of pituitary trophic hormones showed normal basal levels as well as normal responses following stimulation tests No clinical signs of deranged function of the neurohypophysis were observed

Extremely high basal levels of serum C peptide and proinsulin were found during the first 3 years of observation (Table 1) During the arginine infusions and the standardized breakfast test a further increase occurred in both C peptide and proinsulin (Fig 3) No increase in C peptide levels was noted during IVGTT except during the test performed after 4 years of diabetes when a delayed response occurred At the tests 4 and 5 years after onset of diabetes the fasting levels of C peptide had decreased but were still in the upper normal range Proinsulin levels were constantly elevated in the basal state on all occasions Basal glucagon levels were normal and an adequate increase occurred after arginine (Table 1)

Pimozide treatment did not cause any change in the diabetic control (Fig 2), blood lipids or in subcutaneous fat (evaluated by skinfold measurements)

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kinetic, diabetogenic and anti-insulin properties (7, 20, 21)

In the diencephalic syndrome of emaciation a similar loss of subcutaneous fat occurs In this condition, always caused by a brain tumour, high blood levels of growth hormone are found, which are probably responsible for the fat loss (10) In our patient, however, growth hormone levels were not elevated, a finding in accordance with other reports (22, 24)

Insulin and C-peptide are secreted from the B-cell in equimolar amounts (3, 15), and repeated tests in our patient showed grossly augmented levels of C-peptide, indicating highly active B-cells possibly trying to compensate for the hyperglycemia, caused by peripheral resistance to the action of insulin The arginine-induced rise in glucagon levels showed that even the A-cells of the pancreas functioned normally (Table 1) The decrease in C-peptide levels during the 3½ years of observation may be taken as an indication of a gradual B-cell failure The high levels of proinsulin may be due to the presence of insulin antibodies binding large amounts of the proinsulin in complexes and thereby prolonging its half-life However, it cannot be excluded that the B-cell does secrete proinsulin in abnormal amounts in relation to insulin and C-peptide

The cause of the insulin resistance, which is a constant finding in lipoatrophic diabetes, is unknown The relatively low level of insulin antibodies (IgG, Table 1) in our patient cannot explain the insulin resistance, much higher IgG values may be found in insulin-sensitive juvenile-type diabetics (17) Increase concentration of free fatty acids (not investigated in our patient) has been suggested as a pathogenetic mechanism (23), but the basal defect in lipoatrophic diabetes is probably of hypothalamic origin with disturbances in the metabolism of the monoamine neurotransmitters (5, 6 29) Fenfluramine, a blocker of dopamine with the additional effect of lowering brain serotonin levels, lowered the urinary excretion of 5-OH indole acetic acid in liopatrophic diabetes and was found to increase the sensitivity of exogenous insulin in both animals and patients with lipoatrophic diabetes (29, 30) Pimozide, a selective dopamine receptor blocker, has also been shown to induce a return of subcutaneous fat (6). These results, however, have been questioned (28), and in our patient pimozide treatment had no effect, a finding also experienced by others (25, 29, 30)

In accordance with the short term animal experiment in our study (Table 2), urinary peptides from other patients with lipoatrophic diabetes have been found to exhibit anti insulin properties (7) which were not discovered with long-term administration of urinary peptides from our patient The urinary chromatograms of our patient revealed pathological patterns, which were quite different from those obtained in 6 other patients with congenital generalized lipodystrophy, 4 of whom had developed lipoatrophic diabetes (7) Furthermore, the chromatographic patterns varied during the different phases of the disease in our patient, which might reflect different derangements in the diencephalon depending on local pressure variations

The chromatographic pattern obtained before the operation was similar to that observed in patients with cerebral obesity (31), and immediately after the operation similar to that of primary hypothalamic anorexia nervosa (32) Twelve months later the pattern was close to that for normal controls, except for the relatively tall peak corresponding to an elution volume of 700–850 ml This pattern is more typical for 'metabolic' obesity (31)

ACKNOWLEDGEMENT

The excellent assistance of Eva Cornell and Ulla Klingsell is gratefully acknowledged

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Acta Pædiatr Scand 69 545–548, 1980

CASE REPORT

VENTILATORY STUDIES IN TWO OLDER INFANTS WITH PROLONGED APNEA

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ABSTRACT Lagercrantz, H , Broberger, U , Milerad, J and v. Euler, C. (Department of Paediatrics, Karolinska Hospital and the Nobel Institute of Neurophysiology, Karolinska Institute, Stockholm, Sweden) Ventilatory studies in two older infants with prolonged apnea Acta Paediatr Scand 69 545, 1980.—Two infants, both born before term, were found apneic, cyanotic and limp at home when they were 9 weeks and 2 weeks old, respectively Their respiration was monitored in the hospital and found to be remarkably periodic during sleep, and was in one case accompanied by pronounced bradycardia The periodic breathing and apnea seemed to be caused by a decreased oxygen tension which can induce an instability of the central respiratory control mechanisms

KEY WORDS Infants, apnea, periodic breathing, magnetometer, transcutaneous P_{O_2}

Prolonged apnea in older infants has frequently been reported (2, 6 7, 10, 11) This condition is of great interest since it is assumed to be an aborted form of the sudden infant death syndrome although this connection has not been conclusively demonstrated Infants with prolonged apnea have been reported to have impaired reactions to CO_2 (10) and hypoxia (2) or show autonomic instability (6)

We now want to report two patients who had prolonged apnea while at home In Sweden the incidence of the sudden infant death syndrome is low and few cases of so-called near misses have earlier been reported

METHODS

In the first case the respiration was monitored by impedance plethysmography and the heart rate with ECG beat to-beat (Hewlett Packard Paalo Alto Calif USA) In the second case the respiration was monitored with two pairs of linearized infant magnetometers (made by N Peterson Department of Physiology Harvard Medical School Boston Mass USA)

In the second case the transcutaneous P_{O_2} (tcP_{O_2}) was monitored with a Radiometer electrode (Copenhagen, Denmark) The respiration tcP_{O_2} and the ECG were recorded on a Siemens Elema polygraph (type 81 Solna Sweden)

The ventilatory response to CO_2 was tested during periods of quiet sleep The air flow was recorded with a Fleisch pneumotachograph attached to a tightly fitting mask The air flow was integrated to give the volume and recorded on the Siemens Elema polygraph The CO_2 concentration was measured with a capnograph (Gould Godart or Beckman) The infant rebreathed in a small bag (about 2 liters) filled with 100% oxygen until the CO_2 concentration reached about 7% in the bag

The protocol was approved by the local ethical committee

CASE REPORTS

Case 1

This infant boy is the third child to a 33 year-old woman with two healthy older children He was delivered in the 31st gestational week by Caesarean section because of premature rupture of the membranes He was moderately asphyxiated at birth During the first few days he had transient tachypnea and recurrent apnea which was treated effectively with continuous positive air way pressure (CPAP) His condition improved successively and he was discharged home when he was six weeks old He was fed breast milk When he was nine weeks old, after a short period of general discomfort, his parents found him apneic, cyanotic, and limp in his bed during sleep He was resuscitated by mouth to-mouth ventilation and

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Submitted Aug 27, 1979
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DISCUSSION

These two infants had a typical history of the so called near miss sudden infant death syndrome (10, 11) Both were found cyanotic and limp in their beds at home Abnormal frequency and duration of apnea were recorded in the hospital The occurrence of the apnea was associated with upper airway infections

The periodic breathing and the apnea seemed to be correlated with decreased P_{O_2}, possibly caused by the nasal obstruction (11) In both cases the breathing became more regular when 40% oxygen was given In case 2 the periodic breathing could be directly correlated to decreased tcP_{O_2} These findings agree well with those reported by Brady et al (2) who also found that high risk infants became apneic when breathing 17% oxygen

Both infants had a relatively low CO_2 response (202 and 240 ml/kPa kg) The mean response has been reported to be 470±142 (S D) ml/kPa kg (9) and 598±279 ml/kPa kg (1) However the normal range is wide 165–00 ml/kPa kg (1) which we have also seen with our equipment The assessment of the CO_2 response in infants is controversial (9) We cannot conclude whether a decreased CO_2 response is a primary cause of the irregular breathing or due to the prolonged apnea these infants had at home

Theophylline was found to decrease the number of apnea significantly in the first case However no change of the CO_2 response was found Theophylline does not seem to affect the slope of the CO_2 response curve but possibly decreases the threshold of the central chemoreceptors to CO_2 (5)

Cherniack et al (3) have suggested three types of disturbances which can cause periodic breathing in an experimental animal, i) predominant control of breathing by hypoxia ii) overall increase in controller gain and iii) prolonged circulation time In the present cases the first postulated mechanism seems to be most likely the central inspiratory activity is decreased and the respiration is driven primarily by the hypoxic stimulation of the peripheral chemoreceptors Since the relationship between decreased P_{O_2} and the reflex response to this stimulus is hyperbolic, an instability is easily caused Normally, increased CO_2 stabilizes the breathing pattern in this situation (3), but this might not have occurred in these cases due to decreased CO_2 sensitivity

The deep bradycardia in case 1 might also be due to activation of the peripheral chemoreceptors by hypoxia (4 12), but other mechanisms such as vagal reflexes and a pronounced autonomic instability must also be considered (5)

In conclusion these two cases illustrate that some infants with delayed maturation of the central control mechanisms of breathing develop periodic and irregular breathing when P_{O_2} is decreased

ACKNOWLEDGEMENT

Supported by the Swedish Medical Research Council 05234 Expressens Prenatalforskningsnamnd and the Swedish Society of Medical Sciences

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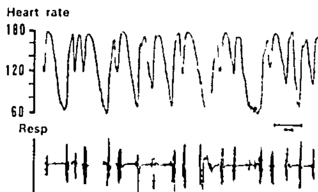


Fig 1 A representative recording of heart rate (beat to beat) and respiration (impedance plethysmography) from case 1 This pattern was seen during periods of 20–60 min that alternated with periods of regular breathing

transferred to the hospital Physical examination of the infant at the hospital did not reveal anything abnormal except a marked muscular hypotonia and nasal discharge Routine analyses of blood electrolytes blood glucose spinal fluid ECG and electroencephalography revealed nothing abnormal Hemophilus influenzae was found in throat culture but blood culture and viral cultures were negative

Respiratory recordings The respiration and heart rate were monitored continuously He was found to breathe periodically (2) or very irregularly with long periods of apnea accompanied by bradycardia (Fig 1) On some occasions he had to be resuscitated by bagging He was breathing 40% oxygen which decreased the number of apneic spells significantly His condition became worse after about 2 weeks (capillary P_{CO_2} increased from 5 2 to 8 1 kPa) and he was given theophylline (3 mg/kg/12 hours) which led to the disappearance of the apnea

When the theophylline treatment was discontinued after two weeks the apnea reappeared Treatment with theophylline was started again and continued for the next nine weeks with satisfactory result

The ventilatory response for CO_2 was tested (when he was 13 weeks old and had not been treated with theophylline for one week) and found to be 202 ml/kPa kg on two occasions No significant change of the slope was found when he was given theophylline No transcutaneous P_{O_2} electrode was available at that time His psychomotor development was initially retarded but he seemed to have developed normally by the time he was 1½ years old

Case 2

This infant girl is the ninth child to a 37 year old woman None of the siblings had any history similar to this She was delivered by Caesarean section in the 37th gestational week due to bleeding and suspected placenta previa She was moderately asphyxiated at birth and developed transient tachypnea She was treated with CPAP during three days and improved successively so she could be discharged home at the age of 10 days

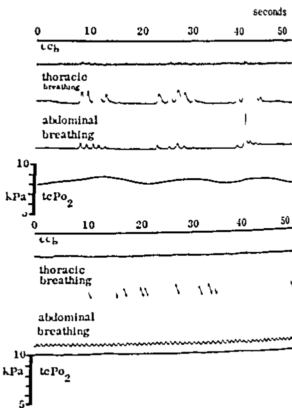


Fig 2 Respiratory recordings (magnetometers) on two levels ECG and transcutaneous P_{O_2} from case 2 Periodic breathing (top) alternated with regular breathing (bottom) during periods of quiet sleep The periodic breathing was mainly seen when the tcP_{O_2} was below 8–9 kPa

Three days later she was found to be apneic limp and cyanotic in her bed about 30 min after feeding

[illegible] tions as in the first case were done but nothing abnormal was found except for an upper airway infection

Respiratory recordings Typical recordings of the respiration monitored with two pairs of magnetometers the ECG and tcP_{O_2} are shown in Fig 2 When the tcP_{O_2} was above 9 kPa during periods of quiet sleep (8) the respiration was regular When she had some difficulties to breathe probably due to nasal obstruction with decreasing tcP_{O_2} (~8 kPa) the breathing pattern became remarkably periodic The breathing was regularized when her breathing became easier and the tcP_{O_2} increased In active sleep the periodic breathing was not seen in spite of low tcP_{O_2}

The fluctuations between regular breathing and periodic breathing in quiet sleep and active sleep could be observed for several hours during the first few days after which the periodic breathing diminished gradually Her capillary P_{CO_2} was only 3 2 kPa and the CO_2 response was 240 ml/kPa kg

She was discharged home at the age of four weeks On follow up examinations her psychomotor development has been normal up to the age of six months

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Submitted Sept 7, 1979
Accepted Nov 30 1979

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Acta Pædiatr Scand 69 549–551, 1980

CASE REPORT

VACCINE ASSOCIATED POLIOMYELITIS IN AN INFANT WITH AGAMMAGLOBULINEMIA

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ABSTRACT Sakano, T , Kittaka, E , Tanaka, Y , Yamaoka, H , Kobayashi, Y and Usui, T (Department of Paediatrics, Hiroshima University Hospital and Hiroshima City Hospital, Hiroshima, Japan) Vaccine-associated poliomyelitis in an infant with agammaglobulinemia Acta Paediatr Scand, 69 549, 1980.—We describe a female infant with agammaglobulinemia who contracted vaccine-associated poliomyelitis Poliovirus type 2 was isolated from the initial stool specimen In our patient, temporary changes in the cerebrospinal fluid resembled those in patients without immunodeficiencies, although gammaglobulin therapy had not yet been started Pleocytosis was observed for a short time after viremia, but soon there was a return to normal without antibody production

Children with antibody deficiency syndrome have an increased susceptibility to bacterial infections but do not seem to have an unusual susceptibility to most viral infections However severe ECHO virus and poliovirus infections have been reported in some patients with humoral or combined immunologic defect (1 2 4 7 8) The present paper describes a female infant with agammaglobulinemia who contracted vaccine associated poliomyelitis

CASE REPORT

Patient K B a 15 month-old baby girl was referred to Hiroshima University Hospital for further evaluation of involuntary movements of the upper limbs She was born of a normal pregnancy and delivery At the age of 10 months the patient contracted a pulmonary infection but made an uneventful recovery She showed almost normal development until the age of 14 months when she was first noted to have progressively less movement of the upper and lower extremities Tremors of the upper limbs and the tongue were also noted Live oral trivalent poliovirus vaccine (OPV) had been administered at the appropriate intervals (Fig 1)

On physical examination bilateral flaccid paralysis of the upper extremities and bilateral foot drop were observed The tendon reflexes of the lower extremities were almost normal on admission Those of the upper limbs were diminished bilaterally Computed tomography of the skull and pneumoencephalography revealed nothing unusual and the electroencephalogram was normal

Cerebrospinal fluid (CSF) showed a slightly elevated cell content predominantly polymorphonuclear Fourteen days later another CSF study showed that all cells were mononuclear and that the total count was normal while protein content had gradually increased (Fig 1) Bacterial cultures of the CSF were negative Viral cultures of the throat and CSF were also negative However poliovirus type 2 was recovered from the initial stool specimen Anti poliovirus antibody was not detected in the patient s serum Antibodies against poliovirus types 1 2 and 3 were demonstrated in the mother s serum There was no family history of susceptibility to infections

IMMUNOLOGICAL STUDIES

The serum IgG content was 0 03 g/l Neither IgM nor IgA was present The number of T lymphocytes in the peripheral blood was normal C3 receptor bearing lymphocytes were present but B lymphocytes bearing surface immunoglobulins were absent In vitro stimulation with phytohaemagglutinin and concanavalin A showed normal ^{3}H [thymidine] incorporation Pokeween mitogen stimulation of the patient s lymphocytes gave a good lymphocyte proliferation as judged from the normal ^{3}H [thymidine] incorporation but blast cells containing immunoglobulins were not demonstrated The lymphocytes isolated from patient s blood when co-cultured with normal peripheral blood lymphocytes showed no suppressor activity on pokeweed mitogen induced B cell differentiation Purified protein derivatives and streptokinase/streptodornase skin tests were nonreactive A skin test with candida

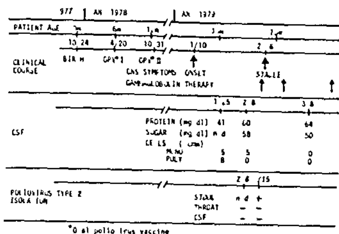


Fig 1 Chronological summary of clinical course

antigen was positive Adenosine deaminase and purine nucleoside phosphorylase activity in erythrocytes was normal Serum Cl_q was 80 1% of the control value Serum hemolytic complement (CH_{50}) was within the normal range

DISCUSSION

The neurologic illness of our patient with agammaglobulinemia was almost certainly due to vaccine-associated poliomyelitis Poliovirus infections following vaccination with live poliovirus have been reported in immunodeficient patients (1, 2, 4, 8) It has been suggested on the basis of clinical data that a combined immunologic defect is necessary for individuals to be at risk of developing severe progressive neurologic disease Suppression of cellular immunity by poliovirus (6) might affect the development of the neurologic illness However, cellular immunity in our patient was grossly intact As described by Wright et al (8), a defect in either branch of immunity may increase the risk of poliomyelitis

In patients such as ours, it can be postulated that administration of OPV induces a viremia without the usual secretory antibody responses in the alimentary tract A viremia at the first administration of OPV might have been dampened by transplacentally acquired maternal antibodies After disappearance of maternal antibodies, the second administration of OPV might have caused severe central nervous system (CNS) involvement

Although our patient had been given trivalent vaccines, poliovirus types 1 and 3 were not isolated Among the immunodeficient patients with vaccine-associated poliomyelitis, poliovirus type 2 has been the most commonly isolated serotype (4, 8) The greater ability of type 2 to cause viremia than types 1 and 3 (3) may be partly responsible for the predominance of type 2 infections

In the CSF of poliomyelitis patients, an early pleocytosis is generally characterized by a predominance of polymorphonuclear cells, but soon the cells are mainly mononuclear and the total count returns to normal Although no gammaglobulin therapy was given during the acute illness, the changes in CSF from our patient showed a common process similar to that of non-immunodeficient patients with poliomyelitis, indicating that once the CNS was invaded, subsequent changes in the CSF were not affected by the presence or absence of antibodies In animal models, the CNS is able to manifest a production of anti poliovirus antibody which is independent of the response in lymphoid tissues (5) However, the possibility of local antibody production in our patient could be excluded, because lymphoid cells responsible for the production of antibody in the CNS have been considered to be mobilized from blood stream (5) In many cases of vaccine-related poliomyelitis, attempts to isolate poliovirus from the CSF have been unsuccessful Failure to isolate poliovirus in the CSF may indicate a transient replication of poliovirus in CSF in contrast to its more prolonged multiplication in the gut Abnormalities in the CSF may be observed for a short time after viremia and return to normal without antibody production

ACKNOWLEDGEMENT

This work was supported in part by a grant in aid from the Japan Medical Research Foundation and by Scientific Research Grants (Project nos 144052 157253 and 467162) from the Ministry of Education Science and Culture of Japan

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Submitted Oct 17 1979
Accepted Jan 11 1980

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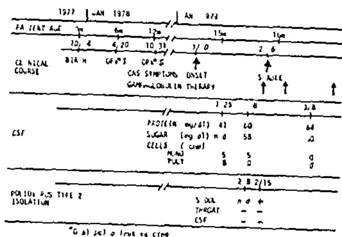


Fig 1 Chronological summary of clinical course

antigen was positive Adenosine deaminase and purine nucleoside phosphorylase activity in erythrocytes was normal Serum Cl_q was 80 1% of the control value Serum hemolytic complement (CH_{50}) was within the normal range

DISCUSSION

The neurologic illness of our patient with agammaglobulinemia was almost certainly due to vaccine-associated poliomyelitis Poliovirus infections following vaccination with live poliovirus have been reported in immunodefi cient patients (1, 2 4, 8) It has been sugge- sted on the basis of clinical data that a combi ned immunologic defect is necessary for indi- viduals to be at risk of developing severe pro- gressive neurologic disease Suppression of cellular immunity by poliovirus (6) might af fect the development of the neurologic illness However, cellular immunity in our patient was grossly intact As described by Wright et al (8), a defect in either branch of immunity may increase the risk of poliomyelitis

In patients such as ours, it can be postulated that administration of OPV induces a viremia without the usual secretory antibody respon- ses in the alimentary tract A viremia at the first administration of OPV might have been dampened by transplacentally acquired mater nal antibodies After disappearance of mater nal antibodies, the second administration of OPV might have caused severe central ner vous system (CNS) involvement

Although our patient had been given tri cnt vaccines, poliovirus types 1 and 3 were isolated Among the immunodeficient patie with vaccine-associated poliomyelitis, polio rus type 2 has been the most commonly isc ted serotype (4, 8) The greater ability of ty 2 to cause viremia than types 1 and 3 (3) m be partly responsible for the predominance type 2 infections

In the CSF of poliomyelitis patients, early pleocytosis is generally characterized a predominance of polymorphonuclear cel but soon the cells are mainly mononuclear a the total count returns to normal Althou no gammaglobulin therapy was given duri the acute illness, the changes in CSF fro our patient showed a common process simil to that of non-immunodeficient patients wi poliomyelitis, indicating that once the CN was invaded, subsequent changes in the CS were not affected by the presence or absenc of antibodies In animal models, the CNS i able to manifest a production of anti polioviru antibody which is independent of the respons in lymphoid tissues (5) However, the possibi lity of local antibody production in our patien could be excluded, because lymphoid cell responsible for the production of antibody ir the CNS have been considered to be mobilize from blood stream (5) In many cases of vac cine-related poliomyelitis, attempts to isolate poliovirus from the CSF have been unsuccess ful Failure to isolate poliovirus in the CSF may indicate a transient replication of poliovi rus in CSF in contrast to its more prolonged multiplication in the gut Abnormalities in the CSF may be observed for a short time after viremia and return to normal without antibody production

ACKNOWLEDGEMENT

This work was supported in part by a grant in aid from the Japan Medical Research Foundation and by Scientific Research Grants (Project nos 144052 157253 and 467162) from the Ministry of Education Science and Culture of Japan

Acta Pædiatr Scand 69 553–557, 1980

CASE REPORT

FULMINANT MENINGOCOCCEMIA IN A CHILD WITH HEREDITARY DEFICIENCY OF THE SEVENTH COMPONENT OF COMPLEMENT AND PROTEINURIA

C LOIRAT D BURIOT A P PELTIER P BERCHE Y AUJARD
C GRISCELLI and H MATHIEU

From the Clinique de Pediatrie Hopital Bretonneau and the U 120 INSERM the U 18 INSERM Centre Andre Lichtwitz Hopital Lariboisiere the Unite d Immunologie et d'Hematologie and the Laboratoire Central de Bacteriologie Hopital des Enfants Malades Paris France

ABSTRACT Loirat, C , Buriot, D , Peltier, A P , Berche, P , Aujard, Y , Griscelli, C and Mathieu, H (Clinique de Pediatrie Hopital Bretonneau and U 120 INSERM U 18 INSERM, Centre Andre Lichtwitz, Hopital Lariboisiere, Unite d Immunologie et d Hematologie and Laboratoire Central de Bacteriologie, Hopital des Enfants Malades, Paris, France) Fulminant meningococcemia in a child with hereditary deficiency of the seventh component of complement and proteinuria Acta Paediatr Scand, 69 553, 1980.—A previously healthy 14-year old boy presented with fulminant meningococcemia He was found to have a total deficiency of C_7 His serum totally lacked bactericidal activity against Neisseria meningitidis Addition of purified C_7 restored the serum hemolytic and bactericidal activity Susceptibility to disseminated Neisseria infections has previously been reported in 3 patients with C_7 deficiency, as well as in a few patients with deficiency of C_5, C_6 and C_8 These findings emphasize the importance of intact complement mediated bactericidal activity in host defense against disseminated Neisseria infections Evaluation of the complement system in individuals with Neisseria infections appears mandatory

KEY WORDS Complement, C_7 meningococcemia

We report the occurrence of a fulminant meningococcemia in a child with complete C_7 deficiency (C D) Disseminated Neisseria infections have already been reported in 3 patients with C D (3 9) and in a few patients with C_5D (20) C_6D (5 8 9 11 21) and C_8D (13) The serum of the propositus totally lacked bactericidal activity notably against *Neisseria meningitidis* This anomaly appears to be found in all sera lacking a normal C_5–C_8 sequence

CASE REPORT

Patient L E was the 14-year-old son of first cousin parents He was hospitalized with fever (40°C) coma cardiovascular collapse and diffuse necrotic purpura Three weeks earlier he had a sore throat with a 3-day penicillin treatment Laboratory findings included hemoglobin 100 g/l leukocyte count 21 8×10⁹/l (74% neutrophils) platelets 45×10⁹/l factor I 0 5 g/l II 30% V 11% VII+X 17% positive fibrin degradation products (32 µg/ml) CSF contained 22×10⁶ cells/l (46% neutrophils) Blood and CSF samples were bacteriologically sterile *Neisseria meningitidis* antigen was present in the urine The clinical course was favorable after intensive resuscitation and ampicillin The coagulation system was within normal limits 4 days after onset Continuous oral penicillin V was prescribed and antimeningococcal (type

found to have proteinuria (1–2 g/l) which persisted during the next 20 years The mother had a kidney biopsy when she was 25 no abnormality was observed on light microscopy A 7 year-old brother of the propositus was in good health In the maternal family several individuals in 4 successive generations were known to have proteinuria They all apparently had a normal life span The paternal family was not available for evaluation

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able 2 *Cell mediated immunity*

ellular activity tested	Assay performed	Patient	Normal values in our laboratory
Delayed hypersensitivity	Tuberculin*	E=10 I=5	E>5 mm I≥5 mm
	Candidin	E=12 I=7	E>5 mm I≥5 mm
T lymphocyte markers in peripheral leukocytes	E RFC*	60%	65±10%
Proliferative response to mitogens	Nonstimulated leukocytes	1 100	100 to 500
	Stimulation with		
	PHA	24 700 (225)[c]	(70 to 200)[c]
	PWM	61 000 (55)	(15 to 40)
	CON A	288 000 (261)	(80 to 200)

Neutrophil functions assessed by chemotactic response to chemotactic factors, phagocytosis of *Staphylococcus aureus* and bactericidal activity were unimpaired

As shown in Fig 1, a total absence of bactericidal activity against *Neisseria meningitidis* type B and *Pseudomonas aeruginosa* 72 VA was observed in the patient's serum (Fig 1) The bactericidal activity was almost completely restored by addition of purified C_7 (Fig 1)

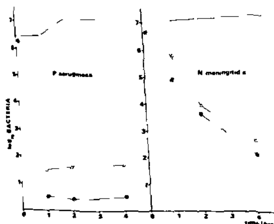


Fig 1 Bactericidal activity of patient serum against *Neisseria meningitidis* serotype B and *Pseudomonas aeruginosa* 72 VA O—O Patient s serum ●—●, pool of normal human sera Δ—Δ patient s serum+purified C_7 Each value is the mean of 3 determinations (S D <0 5)

Renal investigations

There was a permanent 0 60–1 50 g daily proteinuria, which appeared to have a mixed glomerular (selective) and tubular pattern, with β_2 microglobulin excretion The mother's proteinuria had a similar constitution The father s proteinuria was purely glomerular No other anomaly of kidney functions was observed in the propositus Kidney biopsy showed no anomaly on light microscopy Immunofluorescent microscopy showed a few granular deposits with anti C_3 serum fixation along some arteriolar walls No glomeruli were available for immunofluorescence

DISCUSSION

C_7D is a rare condition, transmitted as an autosomal recessive disorder (1, 2, 9, 12, 16, 22), without linkage with the major histocompatibility system (2, 9, 12, 16, 22) To date, 12 cases have been reported In 5, there were no clinical symptoms (2, 7, 9, 22) One female patient had recurrent urinary infections and pyelonephritis (12), which may be a fortuitous association with C_7D Three patients had autoimmune or inflammatory diseases (1, 2, 23) Neisseria infections have been reported as the only clinical problem in 3 cases one patient had meningococcal meningitis and arthritis when 9 years old and disseminated gonococcal

Table 1. *Humoral immunity*

Humoral activity tested	Assay performed	Patient	Normal values in our laboratory
B lymphocyte marker	EAC-RFC[a]	13%	5–25%
Serum Ig production	IgG	1 200	1 050–1 490
	IgM mg/100 ml	190	88–172
	IgA	216	116–228
	IgE IU/ml	317	20–100
Specific antibody production	Allohemagglutinins		
	Anti A	1 32	1 16–1 64
	Anti B	1 64	1 16–1 64

[a] Cells forming rosettes with antibody and complement bound to sheep erythrocytes

METHODS

Functional assays of total hemolytic complement (CH 50) C_1, C_4, C_2, C_8 and C_7 were performed by methods previously described (5, 6, 10) Concentrations of C_1q, C_4, C_3, factor B and C_1 esterase inhibitor were determined by Mancini technique, and concentrations of C_5, C_8 and C_9 by an Ouchterlony test HLA A, B typing of peripheral blood lymphocytes was done by the standard microcytotoxicity assay Antinuclear and antitissue antibodies were determined using an immunofluorescence technique antinative DNA antibodies by the Farr method and circulating immune complexes by the technique of precipitation with polyethylene glycol

Neutrophil chemotactic activity was assayed according to the method of Pham Huu (14) phagocytosis of *Staphylococcus aureus* by neutrophils according to the method of Root & Rosenthal (17) and neutrophil bactericidal activity according to the method of Quie & White (15) The generation of chemotactic activity of the serum was quantified as follows fresh serum (20 μl) was incubated with BSA anti BSA immune complexes in 199 medium for 30 min at 37°C and with zymosan (sigma 0 5 mg/l) for 45 min at 37°C The suspension was heated at 56°C for 30 min to stop complement activation and used as chemotactic factor in a direct observation method (14)

Serum bactericidal assay was performed as follows the assay was performed in Hanks medium with a 20% final concentration The serum of the patient was compared with a pool of normal human sera The bactericidal activity was also tested after addition of purified C_7 component (Cordis Laboratories Miami USA—20% final) to the C_7D serum (20% final) The microorganisms tested were a wild strain of *Neisseria meningitidis* sero type B and a mutant strain of *Pseudomonas aeruginosa* (Strain 72 VA) This strain, known to be very sensitive to complement activity (*unpublished data*), was used as control The final bacterial suspension contained 10^6 to 10^7 bacteria per ml After 1 h, 2 h and 4 h of incubation at 37°C, and gentle agitation, the number of viable bacteria was determined by count on trypticase–soja agar after adequate dilutions in sterile water

Analysis of the proteinuria was performed by immuno electrophoresis and by polyacrylamide gel electrophoresis The kidney biopsy was studied by light and immunofluorescent microscopy

RESULTS

Demonstration of C_7D and HLA typing

The propositus complement system was evaluated on 3 occasions during the year after hospitalisation CH 50 and C_7 activities were *undetectable* The values of the other components were within the normal range Addition of increasing amounts of purified C_7 to the serum resulted in progressive restoration of its hemolytic activity The total hemolytic complement activity of a normal serum was not altered after incubation with equal volumes of the patient's undiluted serum

CH 50 was within normal values in both parents and brother C_7 hemolytic activity was 71% of normal in the father's, 51% in the mother's, and 78% in the brother's serum

HLA determinations were the following propositus HLA A3-B13/AW 29-B12, father HLA A3-B13/A28-B14, mother HLA A1/AW29-B12, brother HLA A28-B14/AW29-B12

Immunological investigations

As shown in Tables 1 and 2, humoral and cell-mediated immunity were normal Antinuclear, antinative DNA and antitissue antibodies, Coombs and Waaler-Rose tests, cryoglobulin and circulating immune complexes were negative

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Submitted Sept 13 1979
Accepted Dec 7 1979

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infection when 29 (9) Two other patients had one (9) or repeated (3) episodes of disseminated meningococcal infection The present observation was characterized by the occurrence of a fulminant meningococcemia and a total C_7D The patient also had an isolated proteinuria since the first months of life, with normal renal parenchyma on light microscopy The proteinuria appeared to be familial and it seems likely that its association with the C_7D was just a coincidence Neisseria disease also appears to be the only type of infection occurring in patients with homozygous deficiencies of C_5, C_6 or C_8 repeated disseminated gonococcal infections have been reported in 3 patients with C_5D (20), C_6D (8) and C_8D (13) respectively, and single (5, 9) or repeated (11, 21) meningococcal meningitis in 4 patients with C_6D

As observed in the present case, C_7 deficient sera display a total lack of bactericidal activity against different gram negative strains (9, 22) In contrast, immunoadherence (12, 22), chemotactic generation (9, 12, 22, present case) and neutrophil phagocytosis (2 9, 12, 22) appear to be normal Total failure of bactericidal activity was also noted in sera of patients with total C_5D (18, 19), C_6D (8 11 21) and C_8D (4, 13) In addition C_5 deficient sera display a failure of chemotactic activity generation (18, 19, 20) These findings suggest that the serum complement mediated bactericidal activity may be important for the host defense against disseminated Neisseria infections (8 9 11, 13) Assessment of CH 50 in patients with disseminated Neisseria infections is obviously mandatory Deficiency of one of the terminal complement components probably justifies a prolonged treatment with penicillin and anti meningococcal vaccination

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Acta Pædiatr Scand 69 559–562, 1980

CASE REPORT

NEONATAL SEPSIS DUE TO GROUP G STREPTOCOCCI

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[illegible] P C Friedman Z , Fairbrother, P F , Hellmann, J and [illegible]

group G streptococci (*Streptococcus canis*) are described [illegible] with transient neonatal hyperthyroidism, while, in the other, concomitant group G streptococcal septicaemia and endometritis in the mother was seen Group G streptococci are rare causes of infection, especially in the paediatric age group Bacteria were identified by serological and biochemical methods Both neonates responded well to penicillin therapy, but the maternal infection required combination therapy with penicillin G, gentamicin, and chloramphenicol The literature on systemic group G streptococcal infection is briefly reviewed With increasing use of serotyping in the identification of β haemolytic streptococci, non-group A organisms will probably be identified more frequently from neonatal and other infections

KEY WORDS Group G streptococci, *Streptococcus canis* neonatal sepsis

Prior to 1970 descriptions of neonatal sepsis due to streptococci were rare (8) However, the past decade has witnessed a dramatic increase in neonatal sepsis due to group B but not other streptococci (3 14 16) Group G streptococci are uncommon causes of infection in the neonatal period (17) and we are aware of only two previous reports of neonatal septicaemia from this cause (2 5) The occurrence at our Institution during a 6-month period of 2 cases of group G streptococcal neonatal sepsis (one accompanied by maternal group G infection) prompted this report The clinical presentation of these patients is discussed and the literature on systemic infection with group G streptococci briefly reviewed

CASE REPORTS

Case 1 A 34-year-old woman (gravida 9 para 5) had Graves disease for 2 years prior to the present pregnancy Initial treatment was with radioactive iodine and propylthiouracil Symptomatic hyperthyroidism reappeared during this pregnancy in spite of increasing the dose of propylthiouracil Maternal T4 levels varied between 182 and 260 nmol/l throughout the present pregnancy

A 2600 g female infant was born via emergency caesarean section for prolapsed cord and foetal distress Apgar scores were 6 and 9 at 1 and 5 min respectively and apart from splenomegaly and inguinal lymphadenopathy she was clinically normal and euthyroid at birth The infant had a few episodes of bradycardia and apnoea during the first 24 hours of life Laboratory investigations on day 1 revealed Haemoglobin 204 g/l Haematocrit 60% peripheral leukocyte count $14\,6\times10^9$/l with 59% neutrophils 22% lymphocytes 16% bands 2% monocytes 1% metamyelocytes platelet count 158×10^9/l serum IgM 0 26 g/l Because of the history of prolonged rupture of the membranes (24 hours) associated with apnoea bradycardia and increased absolute band count ($2\,3\times10^9$/l) cultures of ear nose umbilicus gastric aspirate blood urine and cerebrospinal fluid (CSF) were obtained Overnight culture (sheep blood agar) of all specimens except urine and CSF yielded catalase negative gram positive cocci All isolates were β-haemolytic and bacitracin sensitive (0 04 unit discs) Organisms were serogrouped by the Rantz Randall extraction method (11) using commercially available grouping sera (Wellcome Reagents Beckenham United Kingdom) as well as by the Wellcome Streptex latex ag

ract (2, 17) Most cases of systemic group G streptococcal infection (including septicaemia) have occurred as puerperal sepsis, or in post abortal patients (7, 8, 13, 15) Armstrong et al 1) and Duma et al (8) have described group G streptococcal disease (wound and respira tory tract infections, abscesses, empyema, septicaemia) in adult cancer patients Group G streptococcal endocarditis has been reported (1, 4, 13, 15, 17) and is usually acute, with aggressive course and often serious underlying systemic disease coupled with poor prognosis, in one case these organisms may have played a role in the aetiology of concomitant rheu matic fever (17) Hill et al (13) have de scribed an epidemic of group G streptococcal pharyngitis Other infections with which group G streptococci have been implicated include primary peritonitis lymphadenitis, and pyoderma (4)

Only two previous reports of neonatal septi caemia caused by Lancefield group G strepto cocci could be found in the literature Baker (2) described a premature infant with group G streptococcal sepsis the patient responded satisfactorily to penicillin therapy Brans (5) has reported 2 fatal cases of neonatal septi caemia due to a combination of groups B and G streptococci Baker (2) has emphasized the role of prolonged rupture of the membranes and vaginal colonization with group G organ isms in the pathogenesis of neonatal group G streptococcal sepsis Both of these factors were present in our 2 patients

Our cases of neonatal group G streptococcal sepsis differ from group B infections in the absence of early acute pulmonary/late men ingitic manifestations which usually accom pany infection with the latter organisms (14) In contrast the cases described by Baker (2) and Brans (5) presented with differing degrees of respiratory distress which could have been confused with group B sepsis The observed clinical picture of neonatal group G infection is non specific lack of more data on the clinical presentation of infection with the latter organisms precludes accurate comparison with sepsis due to other organisms at this time

Filker & Monif (11) have postulated an exogenous route for group G streptococcal infection of the female genital tract, especially postpartum The source of maternal post-partum infection in our case is unknown A routine urine culture taken the day before delivery yielded 10^4 colonies/ml of a β haemolytic streptococcus not group A, B, or D Al though grouping by precipitation testing was not carried out, this organism was probably a group G streptococcus Pre operative vaginal cultures were not done We postulate that infection of the mother may have occurred endogenously from organisms either in the lower urinary tract or existing in a situation so as to contaminate a clean catch specimen, i e the vagina, vulva, or introitus Lack of response of maternal infection to penicillin therapy may reflect the presence of other penicillin resistant pathogens which were not grown owing to inadequate culture techniques Group G streptococci are uniformly sensitive to penicillin G (4) and there is thus far no reason to believe that these bacteria are less sensitive to penicillin G *in vivo* than other streptococci, as is the case with group B organisms (16)

Serological cross reaction between group B and G streptococci, which is sometimes seen due to cross reacting carbohydrate antigens (4), was not observed with the current or ganisms in either serogrouping method em ployed This, together with the negative tests for hippurate hydrolysis (Table 1) led us to believe that we were indeed dealing with group G, and not B, organisms (9)

The group G streptococci reported here were β haemolytic and bacitracin sensitive, and could therefore easily have been mis identified as group A streptococci, even though low concentration (0 04 unit) bacitra cin discs have been reported to be highly specific in presumptive identification of the latter organisms (6) Our findings underscore sug gestions by previous workers (2, 4, 15) that,

Table 1 *Reactions of group G streptococci*

Test	Reaction
Haemolysis	β
Growth in 6 5% NaCl	−
Growth on 10% bile agar	+
Growth on 40% bile agar	−
Carbohydrates acid from	
Glucose	+
Maltose	+
Sucrose	+
Galactose	+
Trehalose	+
Glycerol	+
Starch	+
Mannitol	−
Sorbitol	−
Aesculin hydrolysis	−
Gelatin liquefaction	−
Arginine hydrolysis	+
Hippurate hydrolysis	−
Polysaccharide antigens	G[a]

[a] Serogrouping by Rantz Randall autoclave extraction and Streptex latex agglutination methods

glutination method (10) Both methods showed bacteria to be group G streptococci no cross reaction with group B or other streptococcal antisera was observed Identification by biochemical testing (Table 1) was as group G streptococci (4 12) large colony *Streptococcus canis* type (4) Similar organisms were isolated from the mother s vagina The bacteria were sensitive when tested against discs of penicillin G (10 units) methicillin (5 μg) clindamycin (2 μg) erythromycin (15 μg) cephalothin (30 μg) ampicillin (10 μg) and resistant with respect to discs of tetracycline (30 μg) gentamicin (10 μg) kanamycin (30 μg) Initial therapy a combination of ampicillin (50 mg/kg/day) and gentamicin (5 mg/kg/day) was changed to penicillin G (150 000 units/kg/day) when culture and sensitivity results became available

On day 3 the infant became extremely irritable and developed diarrhoea A clinical diagnosis of neonatal thyrotoxicosis was made Because the hyperkinesis and agitation resolved spontaneously by day 6 specific antithyroid treatment was not instituted Thyroid studies on admission revealed T4 273 nmol/l TSH 11 700 μU/l T4 levels increased to 383 5 nmol/l on day 8 but gradually decreased to 306 8 nmol/l on day 12 and 180 7 nmol/l on day 21 TSH levels were 1 100 μU/l on day 8 and 2 400 μU/l on day 21

The infant showed satisfactory weight gain and was discharged in good condition after 10 days of penicillin therapy She was seen again at 3 weeks of age at which time she was clinically euthyroid and well

Case 2 The mother an 18 year old primigravida was admitted at term for observation with ruptured membranes but not in labour She also had a positive glucose tolerance test during pregnancy A caesarean section was performed at full dilatation after a prolonged second stage and a failed attempt at vacuum extraction Maternal temperature was 38 8°C at the time of section and rose to 39 6°C 7 hours post delivery Infection with β haemolytic streptococci was suspected and cultures of blood placenta endometrium wound drainage were taken after section all yielded heavy growth of group G β haemolytic streptococci (*S canis*) with physicochemical characteristics and *in vitro* sensitivity as in Case 1 Laboratory investigation revealed a peripheral leukocyte count of 15 7×10⁹/l with polymorph leukocytosis (78%) Therapy with penicillin G (5 000 000 units intravenously every 4 hours) did not resolve the infection and intravenous chloramphenicol (1 g every 6 hours) and gentamicin (140 mg every 8 hours) were added to the treatment schedule The patient s condition improved on the above regimen and she was discharged in satisfactory condition 11 days after delivery

The foetus weighed 4 200 g Apgar scores were 3 7 9 at 1 5 10 min after birth respectively Physical examination revealed a large occipital haematoma The baby was initially hypotonic but became active and alert shortly thereafter Laboratory investigations yielded peripheral leukocytes 16 2×10⁹/l with neutrophils 3?% lymphocytes 44% bands 10% monocytes 7% eosinophils 3% metamyelocytes 3% basophils 1% Because of ... nose scalp lesion CSF were done all except CSF yielded profuse growth of group G streptococci similar to those isolated from the mother Therapy was with parenteral penicillin G 100 000 units/kg/day for 10 days The baby was discharged after this time in satisfactory condition

DISCUSSION

Neonatal septicaemia may be caused by a wide variety of bacteria the most frequent currently being group B streptococci and coliforms (1 2) The birth canal is an important source of most organisms which cause neonatal sepsis (2 8) In our cases the mode of infection was probably via the mother s vagina during the birth process facilitated by premature rupture of the membranes Additionally in case 2 the possibility of transplacental transmission of infection cannot be excluded

Group G streptococci comprise 2 species a large colony type (*S canis*) and a minute colony type (*S anginosus*) In addition to differences in colonial morphology these organisms differ from each other biochemically (4) Clinical infection with group G streptococci is uncommon especially in children These organisms may be found as normal flora in the throat skin female genital

tract (2, 17) Most cases of systemic group G streptococcal infection (including septicaemia) have occurred as puerperal sepsis, or in post-abortal patients (7, 8, 13, 15) Armstrong et al (1) and Duma et al (8) have described group G streptococcal disease (wound and respiratory tract infections, abscesses, empyema, septicaemia) in adult cancer patients Group G streptococcal endocarditis has been reported (1, 4, 13, 15, 17) and is usually acute, with aggressive course and often serious underlying systemic disease coupled with poor prognosis In one case, these organisms may have played a role in the aetiology of concomitant rheumatic fever (17) Hill et al (13) have described an epidemic of group G streptococcal pharyngitis Other infections with which group G streptococci have been implicated include primary peritonitis lymphadenitis, and pyoderma (4)

Only two previous reports of neonatal septicaemia caused by Lancefield group G streptococci could be found in the literature Baker (2) described a premature infant with group G streptococcal sepsis the patient responded satisfactorily to penicillin therapy Brans (5) has reported 2 fatal cases of neonatal septicaemia due to a combination of groups B and G streptococci Baker (2) has emphasized the role of prolonged rupture of the membranes and vaginal colonization with group G organisms in the pathogenesis of neonatal group G streptococcal sepsis Both of these factors were present in our 2 patients

Our cases of neonatal group G streptococcal sepsis differ from group B infections in the absence of early acute pulmonary/late meningitic manifestations which usually accompany infection with the latter organisms (14) In contrast the cases described by Baker (2) and Brans (5) presented with differing degrees of respiratory distress which could have been confused with group B sepsis The observed clinical picture of neonatal group G infection is non specific lack of more data on the clinical presentation of infection with the latter organisms precludes accurate comparison with sepsis due to other organisms at this time

Filker & Monif (11) have postulated an exogenous route for group G streptococcal infection of the female genital tract, especially postpartum The source of maternal postpartum infection in our case is unknown A routine urine culture taken the day before delivery yielded 10^4 colonies/ml of a β haemolytic streptococcus not group A, B, or D Although grouping by precipitation testing was not carried out, this organism was probably a group G streptococcus Pre operative vaginal cultures were not done We postulate that infection of the mother may have occurred endogenously from organisms either in the lower urinary tract, or existing in a situation so as to contaminate a clean catch specimen, i e the vagina, vulva, or introitus Lack of response of maternal infection to penicillin therapy may reflect the presence of other penicillin resistant pathogens which were not grown owing to inadequate culture techniques Group G streptococci are uniformly sensitive to penicillin G (4) and there is thus far no reason to believe that these bacteria are less sensitive to penicillin G *in vivo* than other streptococci, as is the case with group B organisms (16)

Serological cross reaction between group B and G streptococci, which is sometimes seen due to cross reacting carbohydrate antigens (4), was not observed with the current organisms in either serogrouping method employed This, together with the negative tests for hippurate hydrolysis (Table 1) led us to believe that we were indeed dealing with group G, and not B, organisms (9)

The group G streptococci reported here were β haemolytic and bacitracin sensitive, and could therefore easily have been misidentified as group A streptococci even though low concentration (0 04 unit) bacitracin discs have been reported to be highly specific in presumptive identification of the latter organisms (6) Our findings underscore suggestions by previous workers (2 4, 15) that,

Table 1. *Reactions of group G streptococci*

Test	Reaction
Haemolysis	β
Growth in 6.5% NaCl	–
Growth on 10% bile agar	+
Growth on 40% bile agar	–
Carbohydrates, acid from	
Glucose	+
Maltose	+
Sucrose	+
Galactose	+
Trehalose	+
Glycerol	+
Starch	+
Mannitol	–
Sorbitol	–
Aesculin hydrolysis	–
Gelatin liquefaction	–
Arginine hydrolysis	+
Hippurate hydrolysis	–
Polysaccharide antigens	G[a]

[a] Serogrouping by Rantz Randall autoclave extraction and Streptex latex agglutination methods

glutination method (10). Both methods showed bacteria to be group G streptococci, no cross reaction with group B or other streptococcal antisera was observed. Identification by biochemical testing (Table 1) was as group G streptococci (4, 12), large colony *Streptococcus canis* type (4). Similar organisms were isolated from the mother's vagina. The bacteria were sensitive when tested against discs of penicillin G (10 units), methicillin (5 μg), clindamycin (2 μg), erythromycin (15 μg), cephalothin (30 μg), ampicillin (10 μg), and resistant with respect to discs of tetracycline (30 μg), gentamicin (10 μg), kanamycin (30 μg). Initial therapy, a combination of ampicillin (50 mg/kg/day) and gentamicin (5 mg/kg/day) was changed to penicillin G (150000 units/kg/day) when culture and sensitivity results became available.

On day 3 the infant became extremely irritable and developed diarrhoea. A clinical diagnosis of neonatal thyrotoxicosis was made. Because the hyperkinesis and agitation resolved spontaneously by day 6, specific antithyroid treatment was not instituted. Thyroid studies on admission revealed T4 273 nmol/l, TSH 11200 μU/l. T4-levels increased to 383.5 nmol/l on day 8, but gradually decreased to 306.8 nmol/l on day 12, and 180.7 nmol/l on day 21. TSH levels were 1100 μU/l on day 8 and 2400 μU/l on day 21.

The infant showed satisfactory weight gain and was discharged in good condition after 10 days of penicillin therapy. She was seen again at 3 weeks of age, at which time she was clinically euthyroid and well.

Case 2. The mother, an 18-year old primigravida, was admitted at term for observation with ruptured membranes, but not in labour. She also had a positive glucose tolerance test during pregnancy. A caesarean section was performed at full dilatation after a prolonged second stage and a failed attempt at vacuum extraction. Maternal temperature was 38.8°C at the time of section and rose to 39.6°C 7 hours post-delivery. Infection w[ith] β haemolytic streptococci was suspected and cultures [of] blood, placenta, endometrium, wound drainage w[ere] taken after section; all yielded heavy growth of gr[oup] G β haemolytic streptococci (*S. canis*) with physi[co]chemical characteristics and *in vitro* sensitivity as [in] Case 1. Laboratory investigation revealed a periphe[ral] leukocyte count of 15.7×10⁹/l, with polymorph leu[ko]cytosis (78%). Therapy with penicillin G (5000000 u[nits] intravenously every 4 hours) did not resolve the inf[ec]tion and intravenous chloramphenicol (1 g every 6 hou[rs]) and gentamicin (140 mg every 8 hours) were added to t[he] treatment schedule. The patient's condition improved [on] the above regimen, and she was discharged in sat[is]factory condition 11 days after delivery.

The foetus weighed 4200 g. Apgar scores were 3, 9 at 1, 5, 10 min after birth, respectively. Physical e[x]amination revealed a large occipital haematoma. T[he] baby was initially hypotonic, but became active and al[ert] shortly thereafter. Laboratory investigations yielde[d] peripheral leukocytes 16.2×10⁹/l, with neutrophils 32[%], lymphocytes 44%, bands 10%, monocytes 7%, eosin[o]phils 3%, metamyelocytes 3%, basophils 1%. Because [of] the history of premature rupture of the membrane[s]

profuse growth of group G streptococci similar to tho[se] isolated from the mother. Therapy was with parenter[al] penicillin G 100000 units/kg/day for 10 days. The bab[y] was discharged after this time in satisfactory condition.

DISCUSSION

Neonatal septicaemia may be caused by a wide variety of bacteria, the most frequen[t] currently being group B streptococci and coli[forms] (1, 2). The birth canal is an importan[t] source of most organisms which cause neo[-]natal sepsis (2, 8). In our cases, the mode o[f] infection was probably via the mother's vagina during the birth process, facilitated by premature rupture of the membranes. Additionally, in case 2 the possibility of transplacental transmission of infection cannot be excluded.

Group G streptococci comprise 2 species: a large-colony type (*S. canis*) and a "minute"-colony type (*S. anginosus*). In addition to differences in colonial morphology, these organisms differ from each other biochemically (4). Clinical infection with group G streptococci is uncommon, especially in children. These organisms may be found as normal flora in the throat, skin, female genital

Acta Pædiatr Scand 69 563–566, 1980

CASE REPORT

FIBROMUSCULAR DYSPLASIA IN A CHILD A GENERALIZED ARTERIAL DISEASE

E PESONEN O KOSKIMIES J RAPOLA and J JAASKELAINEN

From the Children s Hospital University of Helsinki Helsinki Finland

ABSTRACT Pesonen, E , Koskimies, O , Rapola, J and Jaaskelainen J (Children s Hospital University of Helsinki, Helsinki, Finland) Fibromuscular dysplasia in a child a generalized arterial disease Acta Paediatr Scand 69 563, 1980.—After slight prodromal symptoms a fourteen-year-old girl had epileptic convulsions followed by a right sided hemiplegia Carotid angiogram showed almost total occlusion of the left arteria cerebri media Six weeks later she developed elevated blood pressure followed by convulsions and lowered consciousness Abdominal aortic angiogram showed total occlusion of the left renal artery at its origin After nephrectomy, the blood pressure returned to normal Histological examination of the main branches of the renal artery revealed fibromuscular dysplastic changes Renovascular hypertension and childhood idiopathic cerebral arterial disease might represent different aspects of the same process.

KEY WORDS Fibromuscular dysplasia, renovascular hypertension, generalized arterial disease, cerebral vascular disease, Moyamoya disease, hemiplegia

The typical localization of fibromuscular dysplasia (FMD) is the renal arteries Other arteries e g the mesenteric (15), the carotid (11) and the intracranial arteries (7 13) as well as those of the lower extremities (12) have also been found to be involved We describe a patient with FMD whose early manifestation was hemiplegia Later it was found that severe changes in renal arteries had been present without any clinical signs of renal ischemia or disease A generalized advanced arterial disease is thus possible in seemingly localized cerebrovascular accidents even without typical symptoms or signs

CASE REPORT

The patient was a 14-year-old previously healthy girl who had been emotionally labile for a couple of weeks After one day s bitemporal headache she had a short attack of aphasia and later during the day a grand mal type of symmetrical epileptiform convulsion with loss of consciousness and a right sided hemiplegia On admission her pulse was 90/min and the blood [illegible])/70 mmHg Hb 156 g/l WBC 7 3×10⁹/l ESR 58 mm/h thrombocytes bleeding and clotting times and serum electrolytes normal screening of virus and toxoplasma titers as well as seroreaction for syphilis negative urine analysis and urine amino acids normal and cerebrospinal fluid analysis normal EEG revealed diminished waves in the left frontal area Left sided carotid angiogram showed nearly total occlusion in the branching of the left arteria cerebri media near the carotid siphon (Fig 1) Collateral circulation via the posterior and anterior cerebral arteries was seen The right sided carotid angiogram displayed obstruction of the frontal branches of the arteria cerebri media and collateral circulation from the arteria pericallosa Computed axial tomography disclosed a wedge shaped area of decreased density in the temporal lobes of both sides

During the following 6 weeks hospitalization her hemiplegia slowly subsided and she was able to walk with support Her systolic blood pressure varied between 110–140 mmHg and her diastolic between 80–90 mmHg until she suddenly developed elevated blood pressure first 150/120 mmHg and later 180/150 mmHg with convulsions and lowered consciousness After this episode of hypertensive crisis the patient was emotionally very labile her muscle strength was decreased and she developed rash on both cheeks and fingertips Phenytoin and prednisone (2 mg/kg/d) were instituted the latter due to suspect collagen disease Immunoglobulins complement fractions C3 and C4 were normal Waaler Rose reaction Latex fixation antinuclear antibodies several tissue specific antibodies and LE cells all turned out to be negative

when β haemolytic bacitracin sensitive streptococci are isolated from systemic infections, identification be confirmed by serogrouping Routine serological identification of β haemolytic streptococci from serious infections is necessary in order to further delineate the role of group G and other streptococci in neonatal and other systemic infections

ACKNOWLEDGEMENTS

We thank Dr Richard R Facklam Head of the Streptococcus Laboratory at the Center for Disease Control Atlanta Georgia for helpful discussion

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Submitted Nov 2 1979
Accepted Dec 12 1979

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Acta Pædiatr Scand 69 563–566, 1980

CASE REPORT

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E PESONEN O KOSKIMIES J RAPOLA and J JAASKELAINEN

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KEY WORDS Fibromuscular dysplasia renovascular hypertension, generalized arterial disease cerebral vascular disease, Moyamoya disease hemiplegia

The typical localization of fibromuscular dysplasia (FMD) is the renal arteries Other arteries e g the mesenteric (15) the carotid (11) and the intracranial arteries (7 13) as well as those of the lower extremities (12) have also been found to be involved We describe a patient with FMD whose early manifestation was hemiplegia Later *it* was found that severe changes in renal arteries had been present without any clinical signs of renal ischemia or disease *A generalized* advanced arterial disease is thus possible in seemingly localized cerebrovascular accidents even without typical *symptoms* or signs

CASE REPORT

aphasia and later during the day a grand mal type of symmetrical *epileptiform* convulsion with loss of consciousness and a right sided hemiplegia On admission her pulse was 90/min and the blood pressure 120/70 mmHg The laboratory tests gave the *following* results Hb 156 g/l WBC 7 3×10⁹/l ESR 58 mm/h thrombocytes bleeding and clotting times and serum electrolytes normal screening of virus and toxoplasma titers as well as seroreaction for syphilis negative urine analysis and urine amino acids normal and cerebrospinal fluid analysis normal EEG revealed diminished waves in the left frontal area Left sided carotid angiogram showed nearly total occlusion in the branching of the left arteria cerebri media near the carotid siphon (Fig 1) Collateral circulation via the *posterior and anterior cerebral arteries* was seen The right sided carotid angiogram displayed obstruction of the frontal branches of the arteria cerebri media and collateral circulation from the arteria pericallosa Computed *axial tomography disclosed a wedge shaped area of* decreased density in the temporal lobes of both sides

During the following 6 weeks hospitalization her hemiplegia slowly subsided and she was able to walk with support Her systolic blood pressure varied between 110–140 mmHg and her diastolic between 80–90 mmHg until she suddenly developed elevated blood pressure first 150/120 mmHg and later 180/150 mmHg with convulsions and lowered consciousness After this episode of hypertensive crisis the patient was emotionally very labile her muscle strength was decreased and she developed rash on both cheeks and fingertips Phenytoin and prednisone (2 mg/kg/d) were instituted the latter due *to suspect* collagen disease Immunoglobulins complement fractions C3 and C4 were normal Waaler Rose reaction Latex fixation antinuclear antibodies several tissue specific antibodies and LE cells all turned out to be negative



Fig 1 Left sided carotid angiogram Nearly total occlusion of the medial cerebral artery (arrow) is present



Fig 2 Abdominal angiogram There is occlusion of the left renal artery (arrow heads) and a small filling defect at the origin of the left renal artery (arrow)

First at this point a proteinuria of 0 35 g/l was detected Because of this and the elevated blood pressure an intravenous pyelography was performed The left kidney did not visualize and the right was hypertrophic over 2 S D above the mean This was interpreted as an indication of compensatory hypertrophy due to longstanding ischemia of the contralateral kidney In the abdominal angiography the left renal artery was totally occluded (Fig 2) and on the right side there were two normal arteries The selective renal vein renin measurements showed high values (160 μg/l/h) on the left side as compared to the right (25 μg/l/h)

A small scarred left kidney was removed The trunk of the left renal artery was occluded Histological examination of the main branches of the left renal artery showed that they were stenosed by intimal myxomatous tissue (Fig 3) The thickness of the media varied from slight thickening to severe hypoplasia The number of elastic fibers in the media was decreased These findings are compatible with the pathologic picture of FMD and especially the intimal and medial form Histological examination of the kidney revealed tightly packed hyalinized glomeruli and tubular atrophy The biopsy taken from the right kidney showed areas of infarctions

After the operation the general condition of the patient slowly improved Prednison as well as antihypertensive medication were gradually discontinued She learned to walk and returned to school seven months after the acute phase Her extremities gained normal functions although on the right side the muscle strength remained at a lower level She is on anticonvulsive medication After our follow up of 12 months the physical findings are normal except a slight right sided weakness

DISCUSSION

FMD is a well known cause of renal hypertension in young adults (5) It may also involve the carotid and vertebral arteries (1 8) The angiographic findings may vary from single stenotic lesion to long segmental stenosis of multiple aneurysms leading to a characteristic beaded appearance This may be similar to that seen in the middle cerebral arteries with childhood idiopathic cerebral arterial disease In the intracranial arterial involvement a direct pathological confirmation may be rare and the diagnosis is based on angiographical findings FMD like angiographic changes have been reported in Moyamoya syndrome (6 10)

The most common clinical symptoms and signs of Moyamoya disease hemiplegia involuntary movements, headache, speech dis

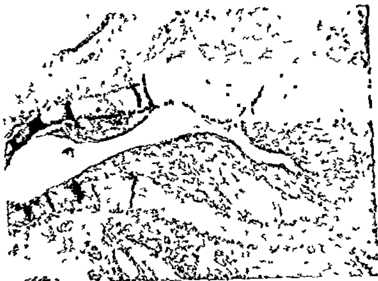


Fig 3 Transverse section of the left renal artery branch Stenosing intimal hyperplasia is present The media is slightly thickened on the left side of the artery ×170

turbances decreased level of consciousness and convulsions (16) were also present in our case Severe hypertension is not a prominent feature of Moyamoya disease but it is noteworthy that in some cases of this disease intimal thickening of the renal arteries has been found (6)

The etiology of FMD is not known Stanley et al (15) have noted that FMD is most prevalent in the arteries which are subjected to mechanical stretching i e the renal artery of mobile kidney the mesenteric arteries and carotid arteries The changes could be interpreted as a repair process of the mechanical arterial damage Intimal fibroplastic changes have been produced experimentally by mechanical (4) immunological (9) and viral stimulus (3) It seems that FMD represents a general tissue response of the arterial wall to various stimuli It has also been suggested to be a congenital developmental disorder (12) A rare case of familial renal artery FMD has been reported (2)

Clinical manifestations of FMD depend on the anatomical location of the stenosing lesion and on the rate of their development At present it is impossible to conclude whether the different clinical syndromes such as Moyamoya disease and renovascular hypertension represent different aspects of the same disease or entirely different vascular diseases

The early progress following the nephrectomy was favorable in our case However infarctions in the remaining kidney indicates the presence of the disease process on that side too The final prognosis which depends on the development of the arterial changes remains unpredictable It has been shown by serial angiograms in adults that progression is present in one third of cases (14)

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Submitted Nov 9 1979
Accepted Dec 15 1979

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Acta Pædiatr Scand 69 567–570, 1980

CASE REPORT

THE AARSKOG SYNDROME

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ABSTRACT. Oberiter, V , Kadrnka Lovrencic, M , Schmutzer, Lj and Kraus, O (Department of Paediatrics and Department of Urology, Dr. Mladen Stojanovic University Hospital, Zagreb, Yugoslavia). The Aarskog syndrome Acta Paediatr Scand, 69 567, 1980.—The Aarskog syndrome is characterized by short stature, peculiar facies, shawl scrotum, cryptorchism, broad, short hands and hyperextensibility of the proximal interphalangeal joints. A boy with typical features of the Aarskog syndrome is presented The proband's mother, sister and grandmother were short and strongly resembled him Palmar dermatoglyphics showed the presence of whorls in the interdigital areas of the affected mother and son and the absence of this pattern on the palms of the sister

KEY WORDS Multiple congenital anomaly (MCA) syndrome, peculiar facies, anomaly of the scrotum, abnormalities of the hands, dermatoglyphics

In 1970 Aarskog described a new syndrome in 7 members from 2 generations of the same family (1) All affected members were short, had a similar facial appearance and genital anomalies Since then, 8 affected families have been reported (2, 4, 11, 12) Two brothers described by Hanley et al (6) in 1967 should be added, as they have a similar pattern of anomalies 9 families with 45 affected members have been described

The affected persons are short Their faces are similar, round in children and triangular in adults, with a short stubby nose, long philtrum, well defined upper lip and pouty lower lip The palpebral fissures have a slightly antimongoloid slant often with unilateral or bilateral ptosis The ears are protuberant with fleshy superior and simple lower helices The hands are broad with short fingers, assuming a peculiar position with hyperextension of the proximal interphalangeal joints Camptodactyly of the distal interphalangeal joints and interdigital webbing of the fingers are often ...rly affected A scrotal skin fold extends ventrally around the base of the phallus like a shawl Cryptorchidism and inguinal herniae are usually present There is a V-shaped midline downward projection of the anterior scalp hair, known as the widow's peak Other anomalies noted include cervical spine abnormalities, sacral spina bifida and osteochondritis dissecans Mild mental retardation has also been described

We present a male patient with the Aarskog syndrome Two female members from 2 gene-

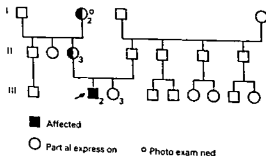


Fig 1 Pedigree of the family



Fig 2 The patient a 15 7/12 year old boy with facial features characteristic of the Aarskog syndrom *ptosis of* the right eyelid widow s peak long philtrum pouty lower lip *thick brows*



Fig 3 Scrotum surrounding phallus frontally There is bilateral cryptorchism

rations of his family display features of this syndrome

CASE REPORT

A 15 7/12 year old boy was admitted for *correction of* cryptorchidism There was no consanguinity (*Fig* 1) Delivery had been uneventful and weight and length normal He went to school regularly but was a *strikingly* poor pupil His height was 158 cm (3rd centile) weight 50 kg (25th centile) His head was brachicephalic with a *circumference of 52 cm He had the typical* facies and his neck was short and broad with *prominent trapezius* muscles The posterior hairline was low and *brest nipples* widely spaced (Fig 2) Hair was absent on the arms and trunk including the axillae present on the legs and pubic hair was stage III (8) The phallus was short and broad The scrotum was of normal size with no prominent raphe and the scrotal fold extended dorsally surrounding the phallus (Fig 3) Three ml testes (10) were found above the external ring with no passage into the scrotal sack Bilateral inguinal herniae were present There was limited extension of the right elbow the thin fingers showed hyperextensibility of the proximal interphalangeal and carpophalangeal joints and flexion of the distal interphalangeal joints There was interdigital webbing between the 2nd and 3rd the 3rd and 5th and the 4th and 5th fingers The toes were long with wide spacing between the 1st and 2nd toes Pedes plani were present He was mildly mentally retarded

Bone age determined according to Greulich & Pyle (5) corresponded to his chronological age

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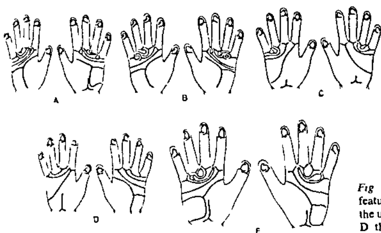


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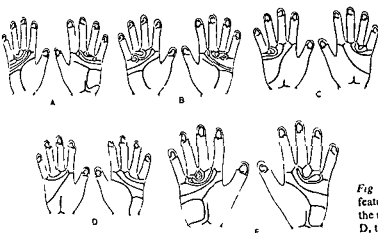


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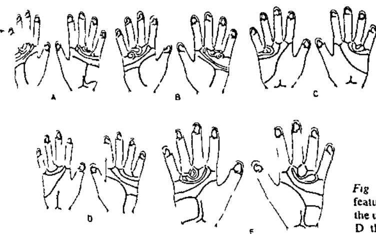


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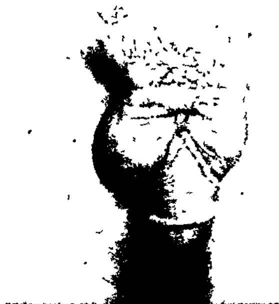


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Acta Pædiatr Scand 69 571–574

CASE REPORT

CONGENITAL HEPATIC FIBROSIS COMBINED WITH PROTEIN LOSING ENTEROPATHY AND RECURRENT THROMBOSIS

P S PEDERSEN and I TYGSTRUP

From the University Clinic of Paediatrics and Laboratory of Pathology Rigshospitalet Copenhagen Denmark

Histopa

bosis s

the second child revealed decreased antithrombin III in plasma, which sugg... genetic implications for the thrombosis tendency seen in both patients

KEY WORDS Congenital Hepatic Fibrosis protein losing enteropathy, thrombosis tendency antithrombin-III deficiency

Congenital Hepatic Fibrosis (CHF) is a rare disorder of the liver caused by maldevelopment of the bile ducts (7) In the familial type of CHF where more than one child in a family is affected kidney changes indistinguishable from those of Potter s type II are a frequent additional finding (7 9 10)

The present report describes the concurrence of two additional features in both of two siblings with CHF namely protein losing enteropathy and thrombosis tendency

CASE REPORTS

The two siblings are the offspring of healthy parents who are related in the respect that the maternal grandparents were first cousins A younger sister who is now 10 years old is in good health No family history of hepatic disorder thrombotic tendency renal or gastrointestinal disease has been recorded

Case I

B K born 13 12 1963 Since the age of 2½ years he had been repeatedly hospitalized because of fever diarrhoea hepatomegaly oedema and tenderness of the legs each time making a spontaneous recovery in a few weeks A gluten free diet for 6 months did not prevent recurrence of the symptoms At the age of 5 he was admitted to Rigshospitalet with the same symptoms and clinical signs of venous occlusion of the left leg After treatment with antibiotics he improved but 2 weeks later he developed signs of venous occlusion of both legs and the left arm together with paralytic ileus Phlebography revealed total occlusion of the iliofemoral veins on the right side and partial occlusion on the left side The v cava was normal Anticoagulation therapy (heparin followed by phenprocumolum) was instituted and the patient improved quickly but venous occlusion reappeared in spite of continuous treatment with phenprocumolum and 2 weeks later he developed severe bronchopneumonia and haematemesis and died within few days despite intensive treatment

Case II

M K born 19 05 1966 (sister of case I) At the age of 2½ years she was hospitalized because of fever vomiting and diarrhoea and in few days she developed hepatomegaly as well as tenderness cyanosis and oedema of the left leg After treatment with heparin followed by phenprocumolum all symptoms abated in a week and she has since remained free of symptoms on continuous phenprocumolum

Laboratory investigations Routine laboratory investigations including liver function tests faecal fat excretion

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Submitted Sept 15, 1979
Accepted Dec 15, 1979

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METHODS

Antithrombin III was determined immunochemically *ad modum* Laurell (11) Platelet adhesiveness was measured *ad modum* Hellem as modified by Cronberg et al (2) Plasminogen was measured by an immunochemical method according to Ekelund et al (4) Inhibition of plasminogen activation by urokinase was measured by the clot method of Paraskevas et al (17) Alpha 2 macroglobulin was determined by an esterolytic method of Ganrot (5) Fibrin/fibrinogen degradation products were measured by the immunochemical method of Nilehn (14) The fibrinolytic activity of plasma and resuspended euglobulin precipitate was measured on unheated fibrin plates according to Nilsson & Olow (15) The fibrinolytic release capacity was measured after venous stasis standardized according to Nilsson & Pandolfi (16) The plasminogen activator activity of the wall of a superficial hand vein was determined by Pandolfi's modification of Todd s fibrinolysis autography technique (16) Additional coagulation tests were done according to the methods

DISCUSSION

The consanguinity in this family together with the known familial occurrence (7, 9 10 19) and the identical case histories support the hypothesis that both cases are suffering from CHF The high priority given to anticoagulation therapy has prevented attempts to establish the histological liver diagnosis in case II A remarkably low serum albumin level due to intestinal loss and decreased synthesis was found but no anatomical or clinical signs seen in connection with protein losing gastroenteropathy (20) was found The only demonstrable abnormality in the coagulation system was an initial low value of AT III in case II

AT III is an alpha 2 globulin known to inhibit the thrombin induced transformation of fibrinogen to fibrin (3) and isolated deficiency of AT-III associated with thrombosis tendency has hitherto been described in five families (3, 8, 12, 13, 18) where the activity of AT-III varied from 26 to 60% of the normal value In case II the values of AT-III were initially decreased (44–52%) but later values, obtained while the patient was free from symptoms and on phenprocumolum therapy, were normal This increase may be an analogous to that in the patients of Marciniak (12) where AT-III rose to more than twice the original value following treatment with phenprocumolum We believe that the relative AT III deficiency, together with a state of hypoalbuminaemia, dehydration and haemoconcentration might have had pathogenetic consequence for the thrombotic episodes in our 2 patients We are aware, however, that a low AT-III does not inevitably give rise to thrombosis, thus, in patients with liver cirrhosis the values are often low, but thrombosis is not a common complication, probably because the synthesis of prothrombin and factor X is simultaneously impaired Our patients did not show decreased values of these coagulation factors and thus resemble those with familial AT-III deficiency

We believe, however, that the AT-III deficiency in our patients represents a secondary phenomenon due to decreased synthesis in the liver and a pathological intestinal loss AT-III, like albumin, is produced in the liver, has a similar molecular weight and is therefore presumably lost the same way

The present report, which describes two new features in CHF, taken together with the frequent combination of CHF with other defects, especially cystic kidney disease, suggests that this rare genetic disorder may in fact be a multiorgan defect

ACKNOWLEDGEMENTS

... e performed by S Jarnum We thank them for their excellent help

Table 1. *Albumin turnover studies*

	Normal	Case I	Case II
[illegible]	35 5–49 9	24 7	19 3
[illegible]	35–53	44	42
[illegible]	37–49	48	67 7
[illegible]	136–241	152	218
[illegible]	7 1–11 2	14 1	26 1
[illegible]			
(*a*) ^{131}I[albumin]	<0 4	1 8	1 7
(*b*) ^{59}Fe[imferon]	<0 8	2 7	3 9

enzyme contents of duodenal juice and tests for specific allergy were all negative Case II showed periodic eosinophilia (max 1085 mill eosinophilocytes/l) Xylose-, glucose-, lactose- and sucrose absorption tests were normal Total serum protein was constantly reduced in both patients (case I 28–62 g/l, case II 25–47 g/l), and serum electrophoresis showed a reduced albumin fraction (case I 20–32 g/l, case II 15–27 g/l) Protein turnover studies revealed a gastrointestinal protein loss in both patients The results of investigations made in periods free from symptoms are shown in Table 1 The albumin synthesis was decreased 50% in case II when a protein turnover investigation was made during one of the acute episodes

Coagulation studies Coagulation tests except for AT III, were normal in both patients, when corrected for anticoagulation therapy The values of AT-III in case II were initially decreased (44–52% of normal value) but then increased gradually in the following years Normal values was achieved when she was 7 years old At this time additional studies were carried out and revealed normal values of platelet adhesiveness, plasminogen activator inhibitors, alpha-2-macroglobulin, fibrin/fibrinogen degradation products, plasminogen activator activity of the vein wall, and fibrinolytic release capacity

Radiographic examination In both patients, oedema of the mucous membrane and precipitation and flocculation of the contrast in the small intestine were seen after barium meal

Liver biopsy (case I) Normal structure except for slight periportal fibrosis

varix?) The liver weighed 1390 g was firmer than normal, and had a uniform fine nodular texture The gall bladder ducts, hepatic veins and the portal vein were all normal The spleen weighed 128 g, and was macroscopically normal The stomach contained haemorrhagic fluid and the intestines were autolytic Liver microscopy showed increased connective tissue of mature collagen type in the portal areas forming broad bands and dividing



Fig 1 Histological picture of the liver in Case I

the hepatic lobules from each other (Fig 1) Many dilated sometimes tortuous bile ducts were seen No inflammatory cells were seen in the connective tissue The arteries and veins were normal In the lobules, normal Kupffer cells and plates of normal hepatocytes were seen Microscopy of the spleen was normal In the iliacal veins a fine reticular connective tissue with recanalized thrombi were found but no thrombi were seen in the arteries The kidneys were found to be normal

METHODS

Antithrombin III was determined immunochemically *ad modum* Laurell (11) Platelet adhesiveness was measured *ad modum* Hellem as modified by Cronberg et al (2) Plasminogen was measured by an immunochemical method according to Ekelund et al (4) Inhibition of plasminogen activation by urokinase was measured by the clot method of Paraskevas et al (17) Alpha 2 macroglobulin was determined by an esterolytic method of Ganrot (5) Fibrin/fibrinogen degradation products were measured by the immunochemical method of Nilehn (14) The fibrinolytic activity of plasma and resuspended euglobulin precipitate was measured on unheated fibrin plates according to Nilsson & Olow (15) The fibrinolytic release capacity was measured after venous stasis standardized according to Nilsson & Pandolfi (16) The plasminogen activator activity of the wall of a superficial hand vein was determined by Pandolfi s modification of Todd s fibrinolysis autography technique (16) Additional coagulation tests were done according to the methods [illegible] scribed by Jarnum et al (6)

DISCUSSION

The consanguinity in this family together with the known familial occurrence (7, 9, 10, 19) and the identical case histories support the hypothesis that both cases are suffering from CHF The high priority given to anticoagulation therapy has prevented attempts to establish the histological liver diagnosis in case II A remarkably low serum albumin level due to intestinal loss and decreased synthesis was found but no anatomical or clinical signs seen in connection with protein losing gastroenteropathy (20) was found The only demonstrable abnormality in the coagulation system was an initial low value of AT III in case II

AT III is an alpha 2 globulin known to inhibit the thrombin induced transformation of fibrinogen to fibrin (3) and [illegible] deficiency of AT-III associated with thrombosis tendency has hitherto been described in five families (3, 8, 12, 13, 18), where the activity of AT-III varied from 26 to 60% of the normal value In case II the values of AT-III were initially decreased (44–52%) but later values, obtained while the patient was free from symptoms and on phenprocumolum therapy, were normal This increase may be an analogous to that in the patients of Marciniak (12) where AT III rose to more than twice the original value following treatment with phenprocumolum We believe that the relative AT-III deficiency, together with a state of hypoalbuminaemia, dehydration and haemo concentration, might have had pathogenetic consequence for the thrombotic episodes in our 2 patients We are aware, however, that a low AT-III does not inevitably give rise to thrombosis, thus, in patients with liver cirrhosis the values are often low, but thrombosis is not a common complication, probably because the synthesis of prothrombin and factor X is simultaneously impaired Our patients did not show decreased values of these coagulation factors and thus resemble those with familial AT-III deficiency

We believe, however, that the AT-III deficiency in our patients represents a secondary phenomenon due to decreased synthesis in the liver and a pathological intestinal loss AT III, like albumin, is produced in the liver, has a similar molecular weight and is therefore presumably lost the same way

The present report, which describes two new features in CHF, taken together with the frequent combination of CHF with other defects, especially cystic kidney disease, suggests that this rare genetic disorder may in fact be a multiorgan defect

ACKNOWLEDGEMENTS

The coagulation analyses were made in the coagulation laboratories in Copenhagen and Malmö by I M Nilsson and V G Nielsen and the protein turnover investigations were performed by S Jarnum We thank them for their excellent help

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Submitted July 17 1979
Accepted Jan 21 1980

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Acta Paediatr Scand 69 575–576, 1980

BOOK REVIEWS

K B Roberts (ed) *Manual of clinical problems in pediatrics with annotated key references* Little Brown and Company Boston Mass 1979 461 pp $10 95 ISBN 0-316-74984-2

This manual is written for medical students and the busy clinician or teacher who want an overview of the basic facts of special pediatric disorders One hundred clinical problems are considered Each section is written by a wellknown specialist and reviewed by a generalist or vice versa All the sections are excellent overviews of the basic facts of the most important pediatric problems More than 2000 references follow each part of the text The references are up to date carefully selected and from well known medical journals

addition the key articles referred to are excellent for the student who wants further knowledge of a particular pediatric disorder

Tomas Sveger

it has been in the work with neurological disorders of childhood It is noteworthy that the authors emphasize that this examination is not the full and only answer to our questions Over and over again they stress the importance of a thorough clinical examination and the avoidance of examinations, which may not be fully justified in diagnosis and treatment This holds for examinations as lumbar puncture airstudies etc

It was a great pleasure for me to read the book and I can recommend it to all paediatric neurological and neurosurgical departments where children with these symptoms or signs are treated But also to other doctors the book contains an amount of information which makes it most valuable in every library It is nicely printed and the pictures are well reproduced Each chapter contains a good list of references

J C Melchior

W E Bell & W F Cormick *Increased intracranial pressure in children* Diagnosis and treatment 2nd edition Vol VIII in the Series Major Problems in Clinical Pediatrics W B Saunders Company Philadelphia London Toronto 1978 485 pp illus £21 00 ISBN 0-7216-1708-5

The book is entirely living up to its titel The authors are clinicians and are really presenting the subject as it should be presented to all of us working in paediatrics neurology neurosurgery radiology and anyone else dealing with children with possible increased intracranial pressure

The division of the book into three major parts makes the reading easy The first part concerns general concepts and gives a detailed discussion of symptoms signs and problems like lumbar puncture when the intracranial pressure is increased roentgenographic signs of increased pressure and of headache in children

The second part of the book which is dealing with etiology is as well written as the rest of the book Of particular interest to the neuropaediatrician is the chapter on pseudotumor cerebri but also the chapters on head trauma and infectious disorders are worthwhile reading

The third part of the book concerns intracranial tumours in childhood and again we are getting an up-to-date survey Of course it is not possible to fulfil all wishes and there might be some dispute concerning classification which could be dealt with otherwise but again the overall impression is an enormous amount of valuable clinical information

The book does not deal much with the medical treatment of intracranial tumours but this is acceptable as

J G Wilson and F C Fraser (eds) *Research procedures and data analysis* Vol IV *In* Handbook of teratology Plenum Press New York 1978 458 pp illus $46 80 ISBN 0 306 36244 9

This fourth and last volume of an extensive handbook dealing with various teratological subjects concentrates on a discussion of usual and unusual laboratory techniques related to teratology—from cytogenetic and mutation testing systems via various in vitro culture techniques to more standard testing techniques for the teratogenic potentials of drugs and other chemicals Most chapters deal with relatively specialized issues but some for instance Wilson s discussion on the feasibility of subhuman primate studies and Rodier s chapter on behavioural teratology perhaps are of more general interest also outside the laboratory The structure of the chapters vary from detailed technical accounts in cookbook style to chapters of a more general discursive nature The final chapter introduces biometrical statistical techniques applied to various problems in teratology laboratory research The scientific standard of all chapters is high the student and researcher in teratology undoubtedly has much to learn from technical descriptions and discussions More detailed descriptions of the actual techniques for the introduction of new students in the field would be an advantage but ample references can always provide the technical information The volume will probably be less interesting to the clinician

Bengt Kallen

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Submitted July 17 1979
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Acta Pædiatr Scand 69 577–580, 1980

LDL RECEPTOR STUDIES IN TERM AND PRE-TERM INFANTS. MEASUREMENT OF STEROL SYNTHESIS IN CORD BLOOD LYMPHOCYTES

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ABSTRACT Andersen, G. E and Johansen, K. B. (The Neonatal Department, Rigshospitalet, University of Copenhagen, Denmark). LDL receptor studies in term and pre-term infants Measurement of sterol synthesis in cord blood lymphocytes. Acta Paediatr Scand, 69 577, 1980.—Low density lipoprotein (LDL) receptor activity was measured in lymphocytes from pre-term and term infants in order to elucidate if the hypercholesterolemia found in pre-term infants might be secondary to a block in cholesterol transport across the cell membrane, analogous to that seen in familial hypercholesterolemia (FH) LDL receptor activity was found to be fully developed in pre-term infants and no different from that of term infants and of a normal adult control

KEY WORDS Hypercholesterolemia, lipoproteins, cholesterol, sterols, lymphocytes, infants

Within the last few years measurement of cord blood lipoproteins has attracted the attention of an increasing number of investigators since it has become clear that hyperlipoproteinemia and especially familial hypercholesterolemia (FH) can express itself at birth by elevated levels of cord serum T-C and LDL-C (1–4) Furthermore several studies have shown that normal (not FH) pre-term newborns have higher cord serum T-C and LDL-C than term infants (5–9). Since LDL (molecular weight around 2 3×10⁶) does not cross the placenta, a maternal source of elevated LDL C in pre-term newborns is very unlikely The present study was made to find out if the mechanism underlying this elevation of serum T-C and LDL-C in normal pre-term babies is analogous to what is seen in patients with FH

Normal cells (fibroblasts, lymphoblasts, lymphocytes, monocytes, arterial smooth muscle cells etc) have been shown to derive the major part of their cholesterol necessary for membrane and hormone synthesis from serum LDL via high affinity LDL cell-surface receptors which regulate intracellular cholesterol synthesis by a feedback regulation mediated specifically by LDL binding to the LDL receptors and degradation of the LDL particles (10–13) If the cell needs more cholesterol the synthesis and thereby the number of LDL receptors will increase and vice versa

In FH the primary genetic defect involves the gene encoding the high affinity LDL receptor Homozygotes have no and heterozygotes only about half normal LDL receptor activity which means that there is a block in cholesterol transport across the cell membrane Homozygotes also have a 6–7 fold and heterozygotes a 2–3 fold increase in serum LDL-C which probably reflects a compensatory reaction to overcome this block

One might imagine that LDL receptor synthesis would be more poorly developed in the fetus during early gestation than at term, i e pre-term newborns would have a smaller

Abbreviations FH familial hypercholesterolemia T C total cholesterol, LDL-C low density lipoprotein cholesterol, LPDS lipoprotein-deficient serum

P H. Morris Jones *Haematology and Oncology Topics in Paediatrics 1*. Pitman Medical, Tunbridge Wells, England 1979, 156 pp , illus £9 50 ISBN 0 272 79540 2

A new series has appeared, "Topics in Paediatrics", and its first issue is devoted to haematology and oncology. The editor P H Morris Jones from the Manchester group is a guarantee for an interesting volume The latest observations as well as fundamental facts in paediatric oncology are well mixed in this very readable book.

In the introductory chapters Birch and Malpas discuss the epidemiology of childhood tumours and its possibilities to provide clues to the etiology of cancer. Most of the chapters concern the management of tumours Special attention is paid to diagnosis and classification, in leukaemia by immunological and cytogenetic analyses, in other tumours by different staging systems The chemotherapeutic regimens are reported in detail and the results are easy to follow in numerous survival tables.

Many of the multidrug treatments show an impressive high survival rate, a result which, according to the editor and many of the authors, can only be achieved by treating the children by multidisciplinary teams Such teams can provide all the supportative cares for the child, have an early awareness of infections and give special attention to the psychological and social problems of the children and their families, who must cope with the terrible uncertainty about whether their child will survive or die It is pointed out that even though these problems are very important few rigorously controlled studies of the emotional consequences of childhood malignancies have been performed

Such a study is underway at the Royal Manchester Children's Hospital It has up to now demonstrated that among the many difficulties these families have to cope with, some could be eliminated or diminished by changed routines and by having a specialist nurse coming home to discuss their worries With more knowledge there might also be other ways of making life easier for the families

This book is highly recommendable to everyone interested in paediatric oncology

Gunilla Berglund

R J Touloukian (ed) *Paediatric trauma* John Wiley & Sons, New York, Chichester Brisbane Toronto 1978 646 pp , illus £24 95, $49 50 ISBN 0 471 01500 8

This is as far as I know the first and only comprehensive textbook on pediatric trauma in a Western language The editor is Professor of Surgery and Pediatrics and Chief of Pediatric Surgery at Yale University School of Medicine, New Haven Many of the 23 contributors are outstanding authorities in their fields

The contents are presented in two parts General Considerations (215 pp) and Specific Injuries (420 pp) A comprehensive index (9 pp) is added Part 1 is introduced by an excellent overview of pediatric trauma stressing the impact of trauma on childhood mortality and morbidity Almost one half of the deaths in the childhood years in the United States (as well as in most other industrialized nations of the world) are a result of trauma A large proportion of these deaths occurs between the scene of the accident and the arrival in the emergency room A comprehensive trauma registry within a protocol suitable for careful evaluation and computerization is suggested in order to improve overall results of transportation, primary and definite treatment The following, equally excellent chapters of Part 1 are dealing with Evaluation and Initial Management, The Psychological Aspects of Physical Trauma, Radiological Considerations, Anesthesia and Intensive Care, Birth Injury, and The Battered Child Syndrome.

Part 2 comprises detailed discussions of the various organ system injuries, dealing with the essentials of their

[illegible]

lot of useful information that a short review of the contents is impossible Each chapter has a comprehensive reference list

The text is clear and concise throughout and amazingly uniform in view of its 23 authors The book is richly and adequately illustrated It is highly recommended to pediatric surgeons, and in particular to general surgeons dealing with pediatric trauma Two important items are missing a second edition ought to include a chapter on the Prevention of childhood accidents, and one on Childhood poisoning

Theodor Ehrenpreis

W B Strong (ed) *Atherosclerosis Its pediatric aspects* Grune & Stratton, New York 1978, 329 pp $17 85 ISBN 0-8089 1113-9

This is an excellent clinically oriented monograph dealing with the pediatric aspects of atherosclerosis, and may serve as a reference source for those interested in the subject Each chapter is written by recognized contributors to particular aspects of atherosclerosis Valuable bibliography accompanies the chapters

The chapters vary considerably in the degree of detail The section on human primate models is far too detailed, which a physiologically oriented student may find interesting but most other readers will find unrewarding The chapters on the biochemical aspects and lipid metabolism might however have benefited from more detail

In spite of these minor drawbacks I recommend this book especially for the pediatrician who is seeking guidance and up to date knowledge about the different aspects of atherosclerosis and its prevention in early life

Tomas Sveger

faximal 'Sppres on	LDL suppres sion	LDL suppres sion (%)
3 73	14 21	52 8
5 34	9 08	69 4
1 53	8 30	68 2
4 37	10 94	62 1
1 19	13 81	63 8
17	19 92	76 2
1 05–9 89	10 63–17 67	61 3–70 9
7		

which the fetus will increase its weight by about 200 g/week and a smaller need for cholesterol during the decelerating growth rate period (37th–40th week of gestation) during which the fetus will increase its weight by only around 70 g/week This need of extra cholesterol in the accelerating growth rate period is reflected by increased levels of serum LDL-C probably synthesized by the liver and avidly taken up by the extra-hepatic cells which regulate the number of LDL receptors as efficiently as at term and in later life as shown by this study A recent study (15) using the same technique as ours in fibroblasts showed that there is no age-related changes in the feedback regulation of cholesterol synthesis when fibroblasts from infants were compared with fibroblasts from 70–81 year old individuals Furthermore Brown et al (16) using a different technique have shown that amniotic fluid cells from a 15- and 18-week fetus have the same LDL receptor activity as fibroblasts from normal adults

In summary there is now substantial evidence that LDL receptor synthesis and regulation of intracellular cholesterol metabolism is fully developed from early fetal life and remains so throughout life in normal individuals.

ACKNOWLEDGEMENTS

This study was supported by grants from the Danish Heart Foundation and the Dagmar Marshall Fund We thank Gitte Femerling Thurmer for preparing the manuscript

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Table 1 *Clinical data and lymphocyte sterol synthesis (dpm/µg lymphocyte protein) in pre term and term newborns*

Sex	Pregnancy	Delivery	Birth weight (g)	Gestational age (weeks)	Maturity	AS/ 1 min	AS/ 5 min	Maximal stimulation
Female	Normal	Normal	3 450	40	AGA	9	10	25 04
Female	Normal	Vacuum extraction (exhausted mother)	4 350	42	LGA	10	10	17 55
Male (twin A)	Normal	Caesarean section (earlier Caesarean section)	3 150	39	AGA	9	10	14 74
Female (twin B)	Normal	Caesarean section (earlier Caesarean section)	2 550	39	SGA	10	10	21 68
Female	An average of 20 cigarettes a day	Pre term uncomplicated	2 300	36	AGA	9	10	29 01
Male	Normal	Pre term uncomplicated	2 130	34	AGA	10	10	41 47
Male Adult control (range of 4 values)								25 38–37 08

capacity to transport cholesterol across the cell membrane (a block) than term infants and thus a secondary rise in serum LDL C to over come this block

To test this hypothesis we therefore investigated LDL receptor activity in pre term and term infants

PATIENTS AND METHODS

The 4 term and 2 pre term newborns are presented in Table 1 The cord was clamped and cut within the first 3 min after delivery Mixed arterial and venous cord blood was collected and contamination with maternal blood carefully avoided Isolation of blood lymphocytes was begun immediately as described earlier (14) LDL receptor activity was evaluated indirectly by measuring cellular cholesterol synthesis under three sets of condition 1) after maximal stimulation of lymphocytes in lipoprotein deficient serum (LPDS) for 68 hours 2) after maximal suppression by adding 7 ketocholesterol after 53 hours in LPDS and 3) after LDL suppression by adding LDL after 53 hours in LPDS Cellular cholesterol synthesis was quantitated by measuring the amount of [2 ^{14}C] in corporated into cellular sterols as described earlier (14) The incorporation of [2 ^{14}C] acetate into [^{14}C] sterols was expressed in dpm/µg cellular protein which was measured as described earlier (14) The day to day variation was assessed by the inclusion of lymphocytes from a healthy male adult These control values are given in Table 1 LDL suppression is expressed as the percentage

$$\frac{(\text{maximal stimulation} - \text{LDL suppression}) \times 100}{\text{maximal stimulation} - \text{maximal suppression}}$$

which reflects the LDL receptor activity If the capacity to synthesize LDL receptors is lower in pre term than in term infants this LDL suppression % would also be lower since the degree of LDL suppression is correlated with the number of LDL receptors

RESULTS

In Table 1 it is seen that the LDL suppression % is of the same order of magnitude in the two pre term infants as in the four term infants Furthermore the level of LDL suppression % is about the same in the newborns and the adult control

DISCUSSION

The study has shown that lymphocytes from pre term infants have a normal capacity to synthesize LDL receptors i e there is no block in cholesterol transport across the cell membrane This means that in pre term infants serum T C and LDL C levels are not elevated by the same mechanism as in FH patients who do have a block

We hypothesize that there is a greater need for cholesterol for membrane and hormone synthesis during the accelerating growth rate period (33nd–36th week of gestation) during

Acta Pædiatr Scand 69 581–585, 1980

SERUM CHOLESTEROL CONCENTRATIONS IN EARLY INFANCY

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ABSTRACT Ginsburg, B E and Zetterstrom R (Department of Paediatrics, Karolinska Institute, St Goran s Children s Hospital, Stockholm, Sweden) Serum cholesterol concentrations in early infancy Acta Paediatr Scand, 69 581, 1980.—Sixteen healthy term infants were followed from birth to the age of 3–6 months Total cholesterol, VLDL-LDL-cholesterol and HDL-cholesterol were determined in cord serum, in serum obtained 3–10 days after birth (mean age 4 6 days) and at the age of 3–6 months (mean 4 1 months) Mean total cholesterol increased by 1 5 mmol/l during the first 3–10 days and by an additional 1 1 mmol/l during the following 3–6 months Mean VLDL-LDL-cholesterol increased by 1 0 mmol/l and 0 9 mmol/l, respectively, and mean HDL-cholesterol by 0 4 mmol/l and 0.3 mmol/l, respectively, during the same periods The HDL-cholesterol VLDL-LDL-cholesterol ratio thus fell from 1.5 at birth to 0 8 at the age of 3–10 days and to 0 6 at 3–6 months In eight breast fed infants, the mean total cholesterol level increased by 2 9 mmol/l from birth to the age of 3–6 months This increase was significantly higher than the increase found in eight infants who received a cow s milk formula—i e 2.3 mmol/l Free and esterified cholesterol were determined in 10 infants Free cholesterol accounted for about one-third of the total cholesterol from birth to the age of 3–6 months

KEY WORDS Total cholesterol, VLDL-LDL-cholesterol, HDL-cholesterol, infants, cow's milk formula breast feeding

Different conditions influence cholesterol homeostasis in different periods of life (1, 2 9) One factor may have an effect at one developmental stage but not at another (2 6) For example a high ratio polyunsaturated/saturated fatty acids (P/S ratio) in the diet lowers the serum cholesterol level in the first year of life but this effect decreases beyond infancy (2 10) The rise in serum cholesterol concentration is rapid during the first days after birth (11 18 19) and then continues slowly during infancy and childhood (1 18)

In the present study we compared the rapid changes in the total cholesterol, HDL cholesterol and VLDL-LDL cholesterol concentrations in the first 3–10 days with the change during the following 3–6 months in the same infant Serum cholesterol values in breast fed infants were compared with those in infants who received supplemental feeding with a

MATERIAL AND METHODS

Of 25 term infants studied in the neonatal period (who data is reported elsewhere (11)) only 16 healthy te infants completed all examinations including a follow at 3–6 months after birth Of the nine infants who we incompletely studied four could not be traced or h moved two parents refused to return and in three th were technical failures at follow up Of the 16 infa who completed the study three were delivered cesarean section The indications for this procedure w contracted pelvis previous cesarean section a psychologic considerations respectively Thirteen had uncomplicated vaginal delivery Cord blood was obtai with the placenta in situ except in the three cases livered by cesarean section where it was obtained imi diately after delivery of the placenta In order to de

Abbreviations T C – total cholesterol VLDL-LDL-C cholesterol in very low density and low density lipop teins HDL-C = cholesterol in high density lipoprotein

This study was accepted by the Ethical Committee of Karolinska Institute Stockholm In accordance with t recommendations informed consent was obtained by of us (B E G) who personally informed the parents al the study and its purpose

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Submitted Febr 15, 1980
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Acta Pædiatr Scand 69 581–585, 1980

SERUM CHOLESTEROL CONCENTRATIONS IN EARLY INFANCY

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ABSTRACT Ginsburg, B.-E and Zetterstrom, R (Department of Paediatrics, Karolinska Institute, St Goran s Children s Hospital Stockholm Sweden) *Serum cholesterol concentrations in early infancy* Acta Paediatr Scand, 69 581, 1980.—Sixteen healthy term infants were followed from birth to the age of 3–6 months *Total cholesterol* VLDL-LDL-cholesterol and HDL-cholesterol were determined in cord serum, in serum obtained 3–10 days after birth (mean age 4 6 days) and at the age of 3–6 months (mean 4 1 months) Mean total cholesterol increased by 1 5 mmol/l during the first 3–10 days and by an additional 1 1 mmol/l during the following 3–6 months Mean VLDL-LDL-cholesterol increased by 1 0 mmol/l and 0 9 mmol/l, respectively and mean HDL-cholesterol by 0 4 mmol/l and 0 3 mmol/l, respectively, during the same periods The HDL-cholesterol VLDL-LDL-cholesterol ratio thus fell from 1 5 at birth to 0 8 at the age of 3–10 days and to 0 6 at 3–6 months In eight breast fed infants, the mean total cholesterol level increased by 2 9 mmol/l from birth to the age of 3–6 months This increase was significantly higher than the increase found in eight infants who received a cow's milk formula—i e 2.3 mmol/l Free and esterified cholesterol were determined in 10 infants Free cholesterol accounted for about one-third of the total cholesterol from birth to the age of 3–6 months

KEY WORDS Total cholesterol, VLDL-LDL-cholesterol HDL-cholesterol, infants, cow's milk formula, breast feeding

Different conditions influence cholesterol homeostasis in different periods of life (1 2 9) One factor may have an effect at one developmental stage but not at another (2, 6) For example a high ratio polyunsaturated/saturated fatty acids (P/S ratio) in the diet lowers the serum cholesterol level in the first year of life but this effect decreases beyond infancy (2 10) The rise in serum cholesterol concentration is rapid during the first days after birth (11 18 19) and then continues slowly during infancy and childhood (1 18)

In the present study we compared the rapid changes in the total cholesterol HDL cholesterol and VLDL-LDL cholesterol concentrations in the first 3–10 days with the change during the following 3–6 months in the same infant Serum cholesterol values in breast fed infants were compared with those in infants who received supplemental feeding with a cow s milk formula

MATERIAL AND METHODS

Of 25 term infants studied in the neonatal period (whose data is reported elsewhere (11)) only 16 healthy term infants completed all examinations including a follow up at 3–6 months after birth Of the nine infants who were incompletely studied four could not be traced or had moved two parents refused to return and in three there were technical failures at follow up Of the 16 infants who completed the study three were delivered by cesarean section The indications for this procedure were contracted pelvis previous cesarean section and psychologic considerations respectively Thirteen had an uncomplicated vaginal delivery Cord blood was obtained with the placenta in situ except in the three cases delivered by cesarean section where it was obtained immediately after delivery of the placenta In order to deter

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Abbreviations T C = total cholesterol VLDL-LDL-C = cholesterol in very low density and low density lipoproteins HDL C = cholesterol in high density lipoproteins

This study was accepted by the Ethical Committee of the Karolinska Institute Stockholm In accordance with their recommendations informed consent was obtained by one of us (B E G) who personally informed the parents about the study and its purpose

Table 1 *Compositions of feedings*

	Breast milk	Cow s milk formula
Fat (g/l)	30–35[b c]	30[a]
Saturated fatty acids (%)	35–45[c]	45
Monounsaturated f a (%)	35–45[c]	40
Polyunsaturated f a (%)	9–16[c]	15
Cholesterol (mg/100 ml)	20–24[c]	2
Protein (g/l)	9[b]	17

[a] Fatty acid pattern C 16=30% C 18=11 5% C 14=7 5% C 18 1=38% C 18 2=15 4%
[b] Lonnerdahl et al (16)
[c] Mellies et al (17)

mine whether this sampling procedure influenced the results two samples were collected in each of two vaginally delivered infants The first sample was obtained within five minutes after clamping the cord and before delivery of the placenta and the second sample immediately after delivery of the placenta No difference was found between the cholesterol values of the two samples

After informed consent was obtained from the parents blood was taken from a peripheral vein of the infant In the three cases delivered by cesarean section this was done 7–10 days after birth and in the remaining 13 infants 3–5 days after birth (mean age 4 6 days) On discharge from the maternity hospital mothers were given general advice and were also asked to note when they started to substitute breast feeding with bottle feeding and when breast feeding was discontinued Bottle fed infants were given a cow s milk formula (the composition is shown in Table 1) An additional venous sample was obtained from a peripheral vein 3 3–5 7 months after birth (mean 4 1 months) Feeding general health weight and height were checked

After clotting in room temperature serum was separated and stored at +4°C The analyses were performed within 7 days of obtaining the samples which is considered to be a duration of storage that does not influence the cholesterol values (13) Total cholesterol was determined by an enzymatic method permitting the estimation of free and esterified cholesterol (21) VLDL LDL cholesterol was determined after precipitating VLDL + LDL with $CaCl_2$/heparin (4) and measuring the cholesterol content of the precipitate HDL cholesterol was calculated as the difference between total cholesterol and VLDL LDL cholesterol

Statistical analysis Non parametric methods were used The significance of the differences was assessed by the Mann Whitney test (22) Correlation coefficients were calculated by the Spearman rank correlation test (23)

RESULTS

T-C, VLDL LDL C and HDL C values in cord blood at the age of 3–10 days and 3–6 months are presented in Table 2 The increase in the mean T-C level was 1 5 mmol/l in the first 3–10 days, and an additional 1 1 mmol/l, in the following 3–6 months Mean VLDL LDL-C increased by 1 0 mmol/l and 0 9 mmol/l, respectively, and mean HDL C by 0 4 mmol/l and 0 2 mmol/l, respectively in the first 3–10 days and in the following 3–6 months The HDL C/VLDL LDL C ratio thus fell from 1 5 at birth to 0 8 at 3–10 days and 0 6 at 3–6 months Eight infants were breast fed throughout the study In the remaining 8 infants, cow's milk formula was also given (Table 1) Bottle feeding was introduced from 1 to 19 weeks before the last examination The mean duration of bottle feeding was about 10 weeks A greater increase in the T-C and HDL C concentration from birth to 3–6 months of age was found in the infants who were only breast fed as can be seen in Table 3 In Table 3 it is seen that current mean serum lipid values were the same in the two groups No correlations between the number of weeks of bottle feeding or weight gain and the increase in the T C concentration were found

There was a significant correlation between the T-C values in cord blood and the values at 3–6 months ($p<0$ 01) Inverse correlations were found between the increase in the first 3–10 days and in the following 3–6 months for the T C values ($p<0$ 01) and the VLDL LDL C values ($p<0$ 01) Free cholesterol accounted for about one third of the T C concentration at all ages during the study (31–35 mean % of T C)

DISCUSSION

There is no agreement on the relation between dietary lipids and cholesterol homeostasis in infancy (2 6, 8, 10, 24) When discussing discrepancies in the results obtained in different studies, one should consider two major factors, namely the age of the infants studied and the composition of their diet No dietary influence on the serum cholesterol level has been demonstrated in infants before

Table 2 *Mean T-C, HDL-C and VLDL-LDL-C values in cord blood and in serum 3–10 days and 3–6 months after birth (n=16)*

	T C mmol/l	HDL-C mmol/l	VLDL LDL-C mmol/l	HDL-C/ VLDL-LDL-C
Cord blood (range)	1 6 (0 7–2 9)	1 0 (0 4–1 9)	0 7 (0 3–1 6)	1 5
3–10 days (range)	3 1 (2 2–4 2)	1 4 (0 8–1 6)	1 7 (0 7–3 6)	0 8
3–6 months (range)	4 2 (3 4–5 7)	1 6 (1 1–2 3)	2 6 (1 5–3 6)	0 6

the age of one week (6, 11, 19) In a free-living population of children on a mixed diet no dietary influence on serum cholesterol level has been demonstrated after the age of one year (2, 3, 10, 12) From the age of six weeks until one year it has been shown that serum cholesterol levels are lower in infants fed a cow's milk formula than in those fed breast milk (6), in infants fed a diet with low fat- and protein content than those on a fat-rich formula (7) and in infants fed a diet with a high P/S ratio than those on a formula with a low P/S ratio (2) According to some authors, cholesterol levels are determined by the total composition of the diet and not by any single ingredient (5, 15) Details about the dietary ingredients are often incomplete, which makes interpretation of the results difficult. The P/S ratio of the diet is probably the most important factor, while the cholesterol content is of minor importance (2, 5) In the present study, 3- to 6-month-old infants who received a cow's milk formula with a cholesterol content that was one tenth of that in human milk, showed a lesser increase in the serum cholesterol concentration than breast fed infants This is in accordance with findings reported by Darmady et al (6) in 6-week old and 4-month-old infants fed a modified cow's milk formula containing 20% linoleic acid In the present study

Table 3 *Data in the 3–6 months-old infants*

	Breast milk only	Supplemental feeding*	
Age (months) (range)	4 1 (3 4–5 7)	4 1 (3 3–5 6)	ns
Weight gain (kg) (range)	2 77 (2 12–4 10)	3 28 (1 44–4 99)	ns
Male/female	3/5	4/4	
Current mean serum lipid values (mmol/l)			
T C	4 5	4 0	ns
HDL C	1 8	1 5	ns
VLDL LDL C	2 7	2 5	ns
Change in mean serum lipid values from cord values (mmol/l)			
T C	2 9	2 3	$p<0\ 05$
HDL-C	0 9	0 4	$p<0\ 05$
VLDL-LDL-C	2 0	1 9	ns

* Supplemental feeding i e cow s milk formula, was introduced 0 9–18 9 (mean 9 7 weeks) before the last examination ns = not significant

however, the current serum lipid values at age 3–6 months were no different in the two groups This finding differs from that reported by Darmady et al (6) and may be caused by a too small number of children

There is good agreement that, although the serum cholesterol level is influenced by diet during the first year of life, this influence does not affect subsequent serum cholesterol levels (2, 10, 12) In the present study, the T-C levels in cord blood correlated with the T-C levels at the age of 3–6 months Andersen et al (2) found no correlation between the T-C levels in cord blood and at the age of 6–10 months or 3–4 years In their study, however, correlations existed between the T-C levels at 3–4 years and those at 7–9 and 14–19 months Potter & Nestel (20) found a weak correlation between the cholesterol concentration at birth and during the second year of life Hardell (14) found no correlation between the T C levels at birth and at the age of one year The correlation between cord and plasma cholesterol concentrations at 5 days of age demonstrated by Potter (19) could not be confirmed by us, most probably because the series was too small These apparently contradictory results might be explained by a correlation between the T C level in cord blood and that in serum during the first months of life A slow alteration in the distribution of the T-C values then occurs Thus the early correlation ceases to exist and a new correlation is present Whether this course is related to changes due to growth and development or to environmental factors or both remains to be determined Andersen et al (2) suggested in their studies that 'serum T C during infancy is under both genetic and dietary influences Beyond infancy, however the dietary influence subsides, whereas the genetic influence seems to persist

During the immediate post natal period serum T C is involved in the metabolic adaptation to extrauterine life and thus the initial change is not determined by the same conditions as the subsequent change

ACKNOWLEDGEMENT

This study was supported by a grant from the Semper Fund for Nutrition Research

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Submitted March 11 1980
Accepted April 15 1980

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Acta Pædiatr Scand 69 587–592, 1980

SERUM CHOLESTEROL CONCENTRATIONS IN NEWBORN INFANTS WITH GESTATIONAL AGES OF 28–42 WEEKS

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ABSTRACT Ginsburg, B E and Zetterstrom, R (*Department of Paediatrics, Karolinska Institute, St Goran s Children s Hospital, Stockholm, Sweden*) Serum cholesterol concentrations in newborn infants with gestational ages of 28–42 weeks Acta Paediatr Scand, 69 587, 1980.—Serum total cholesterol, HDL-cholesterol and VLDL-LDL-cholesterol were determined in 53 newborn infants with gestational ages of 28–42 weeks In pre-term infants (gestational age <37 weeks) the total cholesterol concentration in cord blood was higher than in term infants Mean values were 2 4 and 1 7 mmol/l, respectively The HDL-cholesterol/VLDL-LDL-cholesterol ratio was 1 8 in pre-term and term infants In 11 pre-term and 17 term infants a second determination was made 3–4 days after birth Total cholesterol had increased more in term than in pre-term infants and the difference found at birth had already levelled out Mean value was 3 0 mmol/l in pre-term and term infants The HDL-cholesterol/VLDL-LDL-cholesterol ratio had changed to 0 6 in pre-term and term infants. Six pre-term infants who received intravenous fluids only were also studied Their values did not differ from those in pre-term infants fed orally Free and esterified cholesterol were determined in 26 infants of varying gestational ages About one-third of the total cholesterol was in the free form in pre-term and term infants at birth and during the first days of life

KEY WORDS Total serum cholesterol, HDL-cholesterol, VLDL-LDL-cholesterol, free cholesterol, esterified cholesterol, lipoprotein, newborn, pre term, parenteral nutrition

It is well established that the serum concentrations of lipids and lipoproteins are very low at birth in term infants (1 3 4 7 9 10 13, 14 15 21) In infants with a gestational age <37 weeks the serum concentrations at birth of total cholesterol (T C) HDL cholesterol (HDL C) and VLDL LDL cholesterol (VLDL LDL C) are higher than in term infants (gestational age ≥37 weeks) (1 2 14 19) It is also well established that in term infants there is a rapid postnatal increase in the serum concentrations of lipids and lipoproteins (11, 15 16) Few data are available on the post natal changes in the cholesterol values of the lipoprotein fractions in newborn infants during neonatal adaptation and particularly in pre term infants In the present study we have determined serum concentrations of T C, HDL-C and VLDL-LDL C in pre term and term infants during the first days after birth The ratio free/esterified cholesterol has also been determined

The aim of this study was to ascertain whether the concentration of various serum cholesterol fractions in newborn infants are influenced by gestational age and early postnatal feeding

MATERIAL

Blood samples were obtained from three groups of newborn infants *Group 1* consisted of 24 healthy term infants with a gestational age of 37–42 weeks three of whom were delivered by cesarean section, *Group 2* consisted of 29 pre term infants with a gestational age of 28–36 weeks

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Abbreviations T C=total cholesterol *HDL-C*=high density lipoprotein cholesterol VLDL-LDL-C=very low and low density lipoprotein cholesterol

Table 1. *T-C, HDL-C and VLDL-LDL-C values in cord blood*

	Gestational age	
	<37 weeks (*n*=8)	≥37 weeks (*n*=23)
♂/♀	4/4	11/12
T-C mmol/l		
Mean	2.4**	1.7
Range	1.4–3.4	0.7–2.9
HDL-C mmol/l		
Mean	1.3*	1.0
Range	1.1–1.5	0.5–1.9
VLDL-LDL-C mmol/l		
Mean	1.1$^{n.s.}$	0.7
Range	0.5–2.1	0.3–1.1

n.s. = not significant. * $0.01<p<0.05$. ** $p<0.01$.

seven of whom were delivered by cesarean section. Seven had an Apgar score of 7 or below one minute after birth and 14 had prolonged rupture of the membranes (>24 hours before delivery), and *Group 3* consisted of 6 pre-term infants with a gestational age of 34–36 weeks, who underwent surgery and were maintained on intravenous fluids because of gastrointestinal malformations.

In all infants weight and height were *appropriate for* gestational age. All term infants (Group 1) were nursed by their mothers. Pre-term infants (Group 2) were fed early —i.e. feeding was started within 2–4 hours after *birth*. Human milk was given in rapidly *increasing* amounts of 60, 90, 120 and 150 ml/kg b.w. on the 1st, 2nd, 3rd and 4th days, respectively. The amount retained was checked regularly. Group 3 infants were first given a 10% glucose solution i.v. On the 2nd *postoperative* day (3rd–4th day after birth) they were given an intravenous solution consisting of 10% glucose, Vamin® and Intralipid® in the proportion of 3/2/1. Vamin® is a complete solution of amino acids and Intralipid® consists of emulgated soya oil and egg phospholipids.

EXPERIMENTAL AND ANALYTICAL METHODS

Cord blood was obtained from all infants in Group 1 and from 8 infants in Group 2. The cord was clamped and cut prior to the delivery of the placenta. Mixed arterial and venous blood were allowed to run freely. In three infants arterial and venous cord blood were obtained separately. The cord was double clamped and blood was collected by needle aspiration of the umbilical vein and arteries, respectively. In the remaining 21 infants in Group 2, the first sample was obtained from a peripheral vein within 2–4 hours after birth. In a control study of five infants with gestational ages 32, 33, 33, 33, and 39 weeks, respectively, cord blood and peripheral venous blood was obtained at postnatal ages of 2 h 56, 55, 1 h 44, 1 h 55 and 1 h 24, respectively. In 17 term (Group 1) and 11 pre-term (Group 2) infants another venous blood sample was obtained within the first 3 weeks after birth.

After clotting in room temperature, serum was separated and stored at +4°C. The analyses were done within 4 days of obtaining the samples. Total cholesterol was determined by an enzymatic method (18) permitting the estimation of free and esterified cholesterol. VLDL-LDL cholesterol was determined after precipitating VLDL + LDL with CaCl/heparin (6). The cholesterol content of the precipitate was then measured enzymatically (18). HDL cholesterol was calculated as the difference between total cholesterol and VLDL-LDL cholesterol.

Statistics. The Mann-Whitney test (20) was used to compare cholesterol values in pre-term and term infants and in pre-term infants with perinatal complications and pre-term infants with a normal course.

RESULTS

The concentrations in cord blood of T-C and HDL-C were higher in pre-term infants (gestational age <37 weeks) than in term infants (gestational age ≥37 weeks). VLDL-LDL-C showed the same pattern although the difference was not significant. Mean values and ranges in cord blood are given in Table 1. In three infants with gestational ages of 32, 39 and 40 weeks, respectively, where blood was obtained from the umbilical vein and the arteries, no differences were found. In a control study of four infants it was found that the T-C values were 10–15% higher in the sample obtained from a peripheral vein 1–3 h after

Table 2. *T-C, HDL-C and VLDL-LDL-C values in serum 3–4 days after birth*

	Gestational age	
	<37 weeks (*n*=12)	≥37 weeks (*n*=17)
TC mmol/l		
Mean	3.0$^{n.s.}$	3.0
Range	1.7–4.4	1.9–4.0
HDL-C mmol/l		
Mean	1.1$^{n.s.}$	1.1
Range	0.6–1.8	0.7–1.6
VLDL-LDL-C mmol/l		
Mean	1.9$^{n.s.}$	1.9
Range	1.0–3.2	1.2–2.9

n.s. = *not significant*.

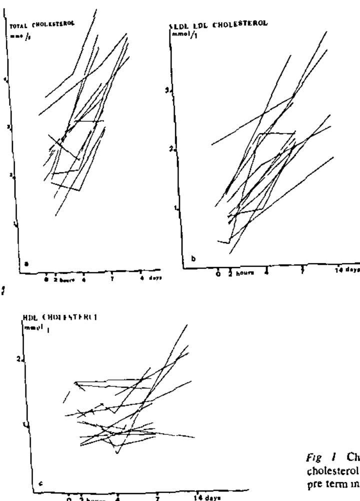


Fig 1 Changes in total cholesterol (*a*) VLDL-LDL-cholesterol (*b*) and HDL-cholesterol (*c*) concentrations in pre term infants 1–2 weeks after birth

birth than in the corresponding cord blood sample

The HDL C/VLDL LDL C ratio in cord blood did not differ in term and pre-term infants the mean values being 1 8 and 1 9, respectively The difference which was found at birth between term and pre-term infants (Table 1) levelled out during the first 3–4 days after birth (Table 2) The HDL C/VLDL LDL C ratio changed during this period to 0 6 in the term and 0 7 in the pre-term infants A few pre term infants were followed for 1–2 weeks T-C increased by about 25% and the HDL C/VLDL-LDL-C ratio was unchanged (0 6 from 5–12 days after birth) (Fig 1)

During the whole period of the study about one third of the T C was in the form of free cholesterol in pre term as well as term infants (Table 3) The newborn infants who were exclusively fed by the i v route during the study showed concentrations of T-C, HDL-C and VLDL-LDL-C in accordance with the values which were found in orally-fed infants (Fig 2) The group, however, was small and heterogenous and did not permit statistical evaluation

Several of the pre term infants, particularly those with a very low gestational age, had perinatal complications In Table 4, the values for three groups of infants with three common complications are given A comparison with

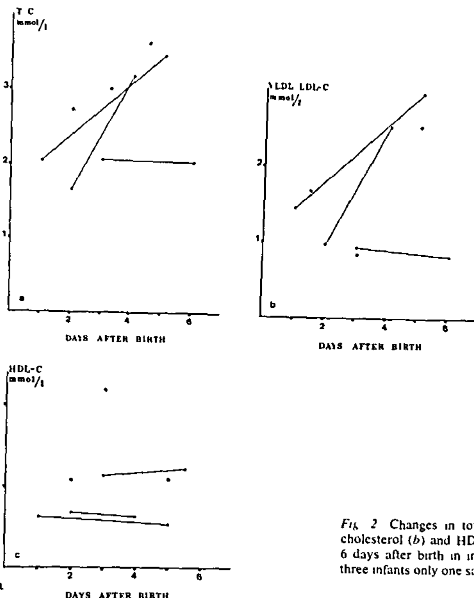


Fig. 2. Changes in total cholesterol (*a*), VLDL-LDL-cholesterol (*b*) and HDL-cholesterol (*c*) during the first 6 days after birth in infants on parenteral nutrition. In three infants only one sample was obtained.

infants having a normal course showed no statistically significant differences. The somewhat lower values found in infants delivered by cesarean section, or those with low Apgar score, might be due to the fact that there was a relatively large proportion of infants with a very low gestational age (<33 weeks). It should be emphasized that the groups compared were small.

DISCUSSION

Cord serum T-C and HDL-C values were found to be higher in infants with a gestational age <37 weeks than in those of a gestational age ≥37 weeks. These findings are in agreement with other recent reports (1, 2, 14, 19) but in conflict with the findings reported by Fosbrooke & Wharton (10) and by Hardell (13). Hardell found a greater influence of

Table 3. *Free cholesterol levels in newborn infants: absolute amounts and relative % of T-C*

	Gestational age					
	<37 weeks			≥37 weeks		
	mmol/l	%	*n*	mmol/l	%	*n*
At birth	0.8	35[n.s.]	15	0.5	32	11
3–12 days	1.4	40[n.s.]	8	1.1	35	11

n.s. = not significant

Table 4 *T C HDL C and VLDL LDL C values at birth in infants with a gestational age of <37 weeks related to some common perinatal complication*

	T C (mmol/l)	HDL C (mmol/l)	VLDL-LDL-C (mmol/l)	Number of infants with a gestational age <33 weeks
Cesarean section (*n*=7)	1 8[n s]	1 2	0 6	4
Apgar score 1 min <7 (*n*=10)	2 1[n s]	1 2	0 9	7
Prolonged rupture of membranes (>24 hours) (*n*=8)	2 5[n s]	1 4	1 0	5
No perinatal complications (*n*=7)	2 2	1 4	0 8	0

n s not significant

sex than of gestational age on T C and VLDL-LDL C levels (13) If cholesterol levels are lower in infants with a very low gestational age (28–33 weeks) the results might be influenced by the proportion of infants with a gestational age <33 weeks Hardell (13) and Fosbrooke & Wharton (10) have included infants with a gestational <33 weeks in their material a fact which might explain why they did not find higher levels in pre term than in term infants The number of data in our study are too few to permit any conclusions Brown et al (5) have found a mean cholesterol concentration of 0 80 mmol/l in four fetuses with gestational ages ranging from 16 to 25 weeks Thus it seems reasonable to suggest that the fetal cholesterol level increases during gestation until a certain age around 32–33 weeks and then falls until term It should be emphasized however that pre term birth is an abnormal state and that findings in pre term infants cannot always be considered to reflect a normal prenatal condition During human pregnancy *maternal* serum cholesterol increases until the 33rd week of gestation (17)

T C HDL C and VLDL LDL C levels were similar in the *umbilical artery* and vein a finding which is in accordance with earlier observations that the placenta does not utilize fetal cholesterol in its biosynthetic pathways and that only small amounts of free cholesterol pass the placental barrier to the fetal circulation (8 12) The human fetus is capable of synthesizing cholesterol (8) Sabata however has reported 15–20% higher cholesterol levels *in the umbilical artery than in the umbilical vein* indicating a fetal output of cholesterol These diverging results might reflect *methodological errors* (19)

HDL C accounted for about 60% of the T C at birth in term as well as pre term infants The ratio HDL-C/VLDL LDL C was 3 2 One third of the T C was in the free, non esterified form not only in term infants, which has been reported earlier (21, 22) but also in pre term infants

Andersen et al (2) recently reported elevated VLDL LDL-C *values in asphyxiated* term infants In the present study, perinatal asphyxia was not found to have any influence on the values There *may be two explanations* for the discrepancy 1) in the present study the asphyxiated infants formed a small heterogeneous group with a *very low* gestational age, 2) some infants with an Apgar *score* at 1 min below 7 were asphyxiated for only a short period Hardell (13) did *not find* any relation between Apgar score and cholesterol *levels*

After birth, there was a rapid change *in the* ratio HDL C/VLDL LDL C from about 1 8 at birth to 0 6 four days later in both term and pre term infants However, the net increase *in* T C was 75% in term infants and 25% in pre term infants The *relative* proportion of free cholesterol remained unchanged around 35% in the whole study

No single mechanism can be responsible for

the complex perinatal changes in HDL C and VLDL C levels The finding that the T-C and VLDL-LDL C levels also increase in newborn infants being on total parenteral nutrition shows that the initial postnatal rise may not solely be due to the feeding of milk, a conclusion which is in accordance with the statement made by Hahn (12)

ACKNOWLEDGEMENTS

This work was supported by grants from the Semper Fund for Nutritional Research The authors are grateful to the staff of the Maternity Department Sabbatsberg Hospital Stockholm for their invaluable cooperation

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Submitted Nov 28 1979
Accepted Febr 28 1980

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Acta Pædiatr Scand 69 593–596, 1980

EVOLUTION OF LIPOPROTEIN PATTERNS IN NEWBORNS

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ABSTRACT Van Biervliet, J P, Vercaemst, R, De Keersgieter, W, Vinaimont, N, Caster, H and Rosseneu, M (Algemeen Ziekenhuis Sint Jan, Brugge, Belgium) Evolution of lipoprotein patterns in newborns Acta Paediatr Scand, 69 593, 1980 —The plasma levels of total and high-density lipoprotein cholesterol and of the major apolipoproteins (apo B and apo A–I) were studied in 30 newborns, on cord blood and after 7 and 30 days of life The evolution of these parameters during the first month of life shows that newborns have low LDL cholesterol and apo B levels at birth which increase drastically during the first week and remain constant between 7 and 30 days The HDL cholesterol and apo A–I levels are proportionally high at birth and keep increasing slowly up to 30 days During this period, the cholesterol/apoprotein ratio remains constant in the LDL and HDL class These data suggest that lipid and apoprotein levels at 7 days are more representative than cord blood levels and more meaningful for a screening of congenital hypercholesterolemia The cholesterol/apo B and apo B/apo A–I ratios, which are considered to be better predictive factors for atherosclerosis, should be included as screening parameters

KEY WORDS Newborns, cholesterol, lipoproteins, apoprotein A–I and B, screening, hypercholesterolemia

Among the known inborn errors of metabolism hypercholesterolemia has one of the highest incidences (6) A large amount of research and epidemiological work (5 12) has been devoted to the study of the plasma lipid patterns in children between 0 and 6 years (2 4) in an attempt to define the optimal parameters and screening procedures for early detection and management of hypercholesterolemia There is increasing evidence that apoprotein levels are better genetic markers than lipids for atherosclerosis (3 11)

In the present study we report the lipid and apoprotein levels in newborns between 0 and 30 days and suggest new approaches to the early detection of lipoprotein abnormalities in infants

SUBJECTS AND METHODS

39 full term babies born after uneventful pregnancy and parturition were studied Cord blood was obtained immediately after clamping of the umbilical cord

Blood was obtained by vena puncture after 7 hours fasting on the morning of the 7th and 30th day after birth and collected in EDTA (1 mg/ml) Any child presenting either medical problems or congenital malformations was discarded from the group The children were either breast fed or bottle fed ad libitum on a humanized formula Given the limited size of the group of newborns the influences of the nutritional parameters are not analysed in this preliminary study

Plasma levels of cholesterol and triglycerides were quantitated by thin layer chromatography with flame ionization detection (13) HDL cholesterol was quantitated by the same technique after precipitation with sodium phosphotungstate (8) Apolipoproteins A I and B were assayed by immunonephelometry using specific antisera (10) The whole procedure required less than 1 ml blood as the plasma and HDL cholesterol quantitation were achieved on 0 2 ml of plasma and both apoproteins were assayed on 0 04 ml plasma

The interassay coefficient of variation amounted to 5% for total cholesterol 7% for HDL cholesterol after precipitation and 6% for the apo A–I and apo B protein The apoproteins were quantitated on a laser Hyland nephelometer by measurement of the relative light scatter after 3 h incubation at 25° with the corresponding antiserum For apo B quantitation a LDL fraction d 1 030–1 053 g/ml was used as standard and the plasma samples were diluted 1/76 For apo A 1 quantitation the plasma was diluted 1/101 with a 6 M GuHCl solution to enable

the complex perinatal changes in HDL C and VLDL C levels The finding that the T-C and VLDL LDL C levels also increase in newborn infants being on total parenteral nutrition shows that the initial postnatal rise may not solely be due to the feeding of milk, a conclusion which is in accordance with the statement made by Hahn (12)

ACKNOWLEDGEMENTS

This work was supported by grants from the Semper Fund for Nutritional Research The authors are grateful to the staff of the Maternity Department Sabbatsberg Hospital Stockholm for their invaluable cooperation

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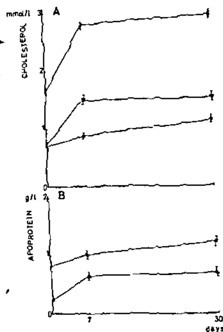


Fig 1 Plasma cholesterol (A) and apoprotein (B) levels in newborns at 0 7 and 30 days expressed in respectively mmol/l and g/l (A) × Total cholesterol ▼ LDL cholesterol ● HDL cholesterol (B) ▲ apo B ■ apo A I

ured at 7 and 30 days For example the correlation coefficient between apo A-I and apo B concentrations at 0 7 and 30 days is −0 05 The LDL and apo B levels increase during the first week of life and remain subsequently constant during one month

DISCUSSION

These data document the changes in plasma lipoprotein concentrations in newborns during the first month of life The results clearly show that the drastic increase in LDL cholesterol observed between 0 and 7 days which had been previously reported (7) is paralleled by an increased concentration of apo B The lipid/apoprotein ratio remains constant

Concerning the HDL class, 60% of their cholesterol and apo A-I levels measured at 30 days are already present at birth (compared to only 35% for LDL) The increase in HDL cholesterol and apo A-I appears as a more progressive and continuous process during the time course of the study At one month, the values are close to those of a normal adult population (3)

From the previous studies, concerned with screening procedures for hyperlipoproteinmia in newborns, it had been suggested that the measurement of total and LDL cholesterol in cord blood should enable the detection of most of the hypercholesterolemic infants (2, 5) Based on the above data, we suggest that apoprotein quantitations could be introduced as important and valuable screening parameters (1) The apo B concentration is a measure of the absolute LDL concentration, while the A-I/B ratio represents the percentage of HDL present in the plasma As HDL is considered as the protective factor against coronary disease (9), while LDL would favor the development of the disease (12), the measurement of both absolute concentrations and ratios would provide a better predictive factor Each apoprotein quantitation by immunonephelometry requires only 20 μl plasma or serum and can be carried out on dried blood

We propose therefore that screening for congenital hypercholesterolemia should be carried out by means of this new method, at the end of the first week of life, as this timing appears the most suitable and best predictive The LDL lipid and apoprotein increase between 0 and 7 days differs strongly from infant to infant and therefore the 7 days value appears to be better related to later concentrations as its represents 50–60% of the adult concentration for LDL cholesterol and apo B in a normal population Such a programme can easily be incorporated in the routine screening procedures, performed for the early detection of inborn errors of metabolism A meaningful interpretation of the results will however require a preliminary study of the possible influence of the diet on the apoprotein and lipid levels at 7 days, in order to establish normal values as a function of the type of diet

Table 1 *Plasma lipids (mmol/l) and apoproteins (g/l) in newborns at days 0 (T_1) 7 (T_2) and 30 (T_3) and evolution of the parameters mean value ± S E M*

	T_1	T_2	T_3	T_2-T_1	T_3-T_2	T_3-T_1
Tot chol	1 67±0 01	2 79±0 01	2 95±0 01	1 12±0 01[a]	0 16±0 15	1 78+0 07[a]
HDL chol	0 77±0 05	0 87±0 05	1 14±0 01	0 39±0 15[a]	0 27±0 1[a]	0 40±0 01[a]
LDL chol	0 75±0 07	1 49±0 01	1 52±0 01	0 72±0 12[a]	0 03±0 1	0 75+0 08[a]
Apo B	0 24±0 03	0 67±0 05	0 70±0 04	0 42±0 04[a]	0 03±0 2	0 46±0 05[a]
Apo A-I	0 84±0 03	1 03±0 05	1 27±0 05	0 19±0 05[a]	0 24±0 05[a]	0 43+0 05[a]
% LDL chol	44±2	53 0±2	51 0±2	9 0±3[b]	−2 0±2	7 0±7[a]
% HDL chol	45 5±2	31 5±1 3	38 7±2	−14 0±3[a]	7 3±2[a]	7+7[a]
Apo A-I/ Apo B	3 5±0 1	1 6±0 1	1 8±0 3	−1 9±1[b]	0 2±1	−1 7+0 5[a]
LDL chol /Apo B	3 2±0 5	2 2±0 2	2 2±0 2	−1 0±1 0	0 0±0 1	−1 0±0 5
HDL chol /Apo A-I	0 89±0 3	0 84±0 3	0 40±0 5	−0 05±2 0	0 06+0 2	0 01+0 1

[a] $p<0.01$ [b] $p<0.05$

maximal exposure of the antigenic sites on the apoprotein Apo A-I isolated by DEAE cellulose chromatography in 6 M urea and lyophilized was used as standard and dissolved in a 6 M GuHCl solution The standard apo A-I concentration was determined by the Lowry procedure (10)

The mean value and standard deviation were calculated for each parameter A Wilcoxon test was used to detect significant differences in the evolution of the parameters at the 0 01 and 0 05 probability levels The correlations between the various parameters and their significance at 1% and 5% levels were tested using the Spearman s rank correlation coefficients

RESULTS

The cholesterol and apoprotein concentrations in plasma and lipoprotein fractions are summarized in Table 1 Plasma—and HDL cholesterol were measured directly and LDL cholesterol calculated by difference according to the approximate formula LDL chol = Tot chol −(HDL chol + Triglycerides/5) (9) This calculated value might be altered by dietary changes affecting the VLDL levels The cholesterol/apoprotein ratio in LDL (cholesterol/apo B) and in HDL (HDL cholesterol/apo A-I) as well as the apoprotein ratios were calculated from the above values The evolution of the lipid and apoprotein concentrations between day 0 7 and 30 is also reported

Table 1 shows that at birth the plasma cholesterol amounts to around 1 7 mmol/l 44% of which is incorporated in the HDL fraction, this correlates with the plasma apoprotein distribution patterns as the level of apo B is low compared to that of apo A-I (0 24 compared to 0 84 g/l) The cholesterol/apoprotein ratio amounts to 3 2 and 0 9 in LDL and HDL fractions Between 0 (T_1) and 7 (T_2) days a drastic increase of total plasma cholesterol and apo B concentrations are observed together with a slight but significant increase in HDL cholesterol and apo A-I levels The increase in total plasma cholesterol reflects mostly that of the LDL cholesterol Apo B increases to a higher extent as the LDL cholesterol/apo B ratio decreases slightly but insignificantly while the HDL cholesterol/apo A-I ratio does not vary significantly As a consequence of the drastic apo B increase the apo A-I/B ratio decreases by a factor 2 2

After 30 (T_3) days the plasma cholesterol concentration does not significantly increase compared to 7 days while the HDL cholesterol keeps increasing slightly During this period the apo B protein does not vary significantly while the apo A-I levels still increase As a result the cholesterol/apoprotein ratio within LDL and HDL is the same at 7 and 30 days The evolution of the lipid and apoproteins depicted in Fig 1 shows that lipid and apoprotein values measured in cord blood do not correlate with the plasma concentrations meas

Acta Pædiatr Scand 69 597–602, 1980

ARTIFICIAL VENTILATION OF PREMATURE NEWBORN RABBITS, EFFECTS OF POSITIVE END EXPIRATORY PRESSURE ON LUNG MECHANICS AND LUNG MORPHOLOGY

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ABSTRACT Nilsson R, Grossmann, G and Robertson, B (Department of Paediatric Pathology and Department of Paediatrics, St Goran s Children s Hospital, Karolinska Institute ... Sweden) Artificial ventilation of premature newborn rabbits, effects of positiv ...
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... PEEP than in controls The application of PEEP also resulted in a significantly
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parenchyma

KEY WORDS Artificial ventilation bronchiolar epithelial lesions, hyaline membranes, IRDS lung compliance, newborn rabbits, positive end expiratory pressure

Bronchiolar epithelial lesions similar to those seen in the idiopathic respiratory distress syndrome (IRDS) can be induced in premature newborn animals by artificial ventilation (1 4 12 13 14 15 16 19) It therefore seems likely that patients with severe IRDS subjected to artificial ventilation risk having the respirator treatment aggravate epithelial lesions that belong to the natural course of the disease This may in turn lead to the late complication known as bronchopulmonary dysplasia a chronic disorder characterized by squamous metaplasia in conducting airways, emphysema and interstitial fibrosis Studies by Taghizadeh & Reynolds (21) show that the degree of bronchiolar epithelial lesions in bronchopulmonary dysplasia is indeed correlated to the peak pressure applied during the period of artificial ventilation

Ventilation with positive end expiratory pressure (PEEP) is superior to conventional respirator treatment in the management of severe IRDS in human babies and apparently reduces the risk of bronchopulmonary dysplasia (2) suggesting that the use of PEEP might prevent the exacerbation of epithelial lesions by artificial ventilation We have tested this hypothesis experimentally in premature newborn rabbits ventilated under standardized conditions, with or without PEEP, by assessing epithelial lesion development morphometrically

MATERIAL AND METHODS

Animals

44 premature newborn rabbits removed on day 27 of

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and 5 ml potassium chloride (150 mg/ml) The abdomen

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Submitted Febr 5 1980
Accepted April 9, 1980

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Table 2 *Insufflation pressure (P_I), lung–thorax compliance (C), alveolar expansion index (I_a) and index for bronchiolar epithelial lesions (I_b) in premature rabbit neonates, delivered on day 27 of gestation*

The observations were made after 10 min ventilation with standardized tidal volume (10 ml/kg) with or without PEEP (5 cm H_2O) Values are given as $\bar{X}$+S D

	Without PEEP (n=12)	With PEEP (n=12)	p<
P_I (cm H_2O)	34±6	22±4	0 002
C (ml/cm H_2O kg)	0 35±0 09	0 53±0 11	0 002
I_a	0 59±0 15	0 67±0 18	NS
I_b	0 16+0 14	0 04±0 07	0 02

Table 4. *Maximal inspiratory and expiratory air flow and inspiratory air flow pattern in premature rabbit neonates, delivered on day 27 of gestation*

The observations were made after 10 min ventilation with standardized tidal volume (10 ml/kg), with or without PEEP (5 cm H_2O) Values are given as $\bar{X}$ ±S D

	Without PEEP (n=12)	With PEEP (n=12)	p<
Maximal inspiratory air flow (ml/sec)	1 76±0 28	1 56±0 19	NS
Maximal expiratory air flow (ml/sec)	1 25±0 21	0 94±0 17	0 002
Biphasic inspiratory air flow pattern	9/12	0/12	0 001

ed with PEEP This is true even if the PEEP level is included in the insufflation pressure In other words, the compliance of the lung–thorax system was increased following application of PEEP (Table 2, Fig 1)

Similar observations were made in the two groups of fetuses ventilated with standardized P_I Thus, at the end of ventilation, V_T was significantly greater in animals treated with PEEP, reflecting increased compliance of the lung–thorax system (Table 3)

Measurements of air flow in fetuses ventilated with standardized V_T reveal that maximal expiratory flow was reduced in fetuses treated with PEEP. However, application of PEEP did not cause any significant change in maximal inspiratory flow (Table 4). In 9 of the 12 fetuses ventilated without PEEP, the inspiratory flow tracing showed a biphasic pattern, i e there was a brief period of low, constant flow shortly after the onset of inspiration, followed by accelerated inspiratory flow. This caused a characteristic "hump" in the flow tracing during early inspiration (Fig 2) In the three remaining fetuses ventilated without PEEP the flow tracings showed linear

Table 3 *Tidal volume (V_T) lung–thorax compliance (C) alveolar expansion index (I_a) and index for bronchiolar epithelial lesions (I_b), in premature rabbit neonates delivered on day 27 of gestation*

The observations were made after 10 min ventilation with standardized insufflation pressure (35 cm H_2O) with or without PEEP (5 cm H_2O) Values are given as $\bar{X}$ ±S D

	Without PEEP (n=10)	With PEEP (n=10)	p
V_T (ml/kg)	12 62±8 78	24 87±5 88	<0 01
C (ml/cm H_2O kg)	0 36±0 25	0 69±0 16	0 01
I_a	0 42±0 11	0 66±0 35	NS
I_b	0 12±0 07	0 06±0 05	<0 05

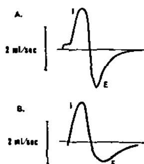


Fig 2 Recordings of air flow in two premature rabbit neonates after 10 min ventilation with standardized tidal volume (10 ml/kg), without PEEP (A) and with PEEP (5 cm H_2O) (B) Body weight in both fetuses was 31 g

ow is low I=inspiration, E=expiration

Table 1 *Survey of the material*

Treatment	Number of neonates	Body weight (g $\bar{x}\pm$S D)
1 Standardized V_T (10 ml/kg) without PEEP	12	33±4
2 Standardized V_T (10 ml/kg) with PEEP (5 cm H_2O)	12	31±4
3 Standardized P_I (35 cm H_2O) without PEEP	10	34±2
4 Standardized P_I (35 cm H_2O) with PEEP (5 cm H_2O)	10	34±5

was immediately opened the vessels of the uterus were clamped with a large hemostat and the fetuses removed after hysterotomy After birth the fetuses received intraperitoneal injections of 0 1 ml sodium pentobarbital (6 mg/ml) and 0 1 ml pancuronium bromide (Pavulon® 0 2 mg/ml Organon Holland) They were tracheotomized kept at 37°C in a volume constant body plethysmograph and connected to a Harvard Rodent Ventilator 680 set at a frequency of 60/min During the period of artificial ventilation the fetuses were fixed in dorsal position by ECG electrodes through their paws Only fetuses showing ECG evidence of cardiac activity throughout the period of ventilation were included in the study

Ventilation

Insufflation pressure (P_I) and pressure changes in the plethysmograph were registered simultaneously by means of pressure transducers (EMT 33 and 34 Siemens Elema Stockholm Sweden) and recorded on a Mingograph 81 (Siemens Elema)

The fetuses were divided into four groups (Table 1)

1 Ventilation with standardized tidal volume (V_T= 10 ml/kg) without PEEP

2 Ventilation with standardized V_T (10 ml/kg) and PEEP (5 cm H_2O)

3 Ventilation with standardized P_I (35 cm H_2O according to our previous experience this pressure level is usually required to maintain V_T in the range of 10 ml/kg in control fetuses at this day of gestation) (13) without PEEP

4 Ventilation with standardized P_I (35 cm H_2O) and PEEP (5 cm H_2O)

In groups 1 and 2 air flow was recorded with a differential pressure transducer (EMT 32C Siemens Elema) connected to the plethysmograph via a specially designed Fleisch tube (11)

All animals were ventilated with air Ventilation was limited to 10 min as epithelial lesions develop in control fetuses within this period (14)

Histologic and morphometric techniques

After artificial ventilation the fetuses were killed by intraperitoneal injection of Mebumal vet ® (0 5 ml) The chest of the fetus was opened and a cannula inserted into the pulmonary trunk The lungs were insufflated at a pressure of 35 cm H_2O The endotracheal pressure was then lowered to 15 cm H_2O this pressure was maintained during the subsequent perfusion The pulmonary artery was perfused for 15 min with a mixture of 1% glutaraldehyde and 3 5% formaldehyde at a pressure of 65 cm H_2O After perfusion the lungs and the heart were left in situ and kept in 10% formaldehyde for at least 24 hours

Paraffin sections from the basal portions of the lungs were stained with hematoxylin and eosin and examined microscopically The relative volumes of the alveolar spaces (V_a) and solid parenchyma (V_p including inter alveolar septa vessels bronchial and bronchiolar walls) were determined by point counting (3) The alveolar expansion index (I_a) was calculated by the formula

$$I_a = \frac{V_a}{V_p} \quad (6)$$

The same sections were reexamined in high power fields (HPFs) and the number of HPFs showing evidence of bronchiolar epithelial necrosis were counted and related to the total number of HPFs examined according to the formula

$$I_b = \frac{\text{no of HPFs with lesion}}{\text{no of HPFs examined}} \times I_a \quad (13)$$

The alveolar expansion index is used here as a correction factor to compensate for variations in the volume of the alveolar compartment that might influence the spacing of the bronchioles The Wilcoxon two-sample two tailed test and the Chi square test were used for statistical comparison

RESULTS

Comparison of results in groups 1 and 2 shows that the standardized V_T was maintained with a significantly lower pressure in fetuses treat

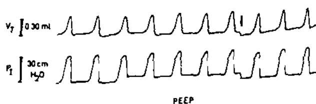


Fig 1 Recordings of tidal volume (V_T) and insufflation pressure (P_I) in premature rabbit neonate (body weight 31 g) during positive pressure ventilation with a short period of PEEP (5 cm H_2O) the setting of the ventilator pump is constant during this phase of the experiment During the PEEP period V_T increases although the effective insufflation pressure decreases reflecting increased compliance of the lung–thorax system (from 0 30 ml/cm H_2O kg to 0 35 ml/cm H_2O kg during PEEP) The base line shift at the end of the PEEP period (arrow) corresponds to a reduction of FRC of approximately 0 04 ml

Table 2 *Insufflation pressure (P_I), lung–thorax compliance (C) alveolar expansion index (I_a) and index for bronchiolar epithelial lesions (I_b) in premature rabbit neonates delivered on day 27 of gestation*

The observations were made after 10 min ventilation with standardized tidal volume (10 ml/kg) with or without PEEP (5 cm H_2O) Values are given as $\bar{x} \pm$ S D

	Without PEEP (n=12)	With PEEP (n=12)	p<
P_I (cm H_2O)	34±6	22±4	0 002
C (ml/cm H_2O kg)	0 35±0 09	0.53±0 11	0 002
I_a	0.59±0 15	0 67±0 18	NS
I_b	0 16±0 14	0 04±0 07	0 02

Table 4 *Maximal inspiratory and expiratory air flow and inspiratory air flow pattern in premature rabbit neonates, delivered on day 27 of gestation*

The observations were made after 10 min ventilation with standardized tidal volume (10 ml/kg) with or without PEEP (5 cm H_2O) Values are given as $\bar{x} \pm$ S D

	Without PEEP (n=12)	With PEEP (n=12)	p<
Maximal inspiratory air flow (ml/sec)	1 76±0 28	1 56±0 19	NS
Maximal expiratory air flow (ml/sec)	1 25±0 21	0 94±0 17	0 002
Biphasic inspiratory air flow pattern	9/12	0/12	0 001

ed with PEEP This is true even if the PEEP level is included in the insufflation pressure In other words, the compliance of the lung–thorax system was increased following application of PEEP (Table 2, Fig 1)

Similar observations were made in the two groups of fetuses ventilated with standardized P_I Thus, at the end of ventilation, V_T was significantly greater in animals treated with PEEP reflecting increased compliance of the lung–thorax system (Table 3)

Measurements of air flow in fetuses ventilated with standardized V_T reveal that maximal expiratory flow was reduced in fetuses treated with PEEP. However, application of PEEP did not cause any significant change in maximal inspiratory flow (Table 4) In 9 of the 12 fetuses ventilated without PEEP, the inspiratory flow tracing showed a biphasic pattern, i e there was a brief period of low, constant flow shortly after the onset of inspiration, followed by accelerated inspiratory flow This caused a characteristic "hump" in the flow tracing during early inspiration (Fig 2) In the three remaining fetuses ventilated without PEEP the flow tracings showed linear

Table 3 *Tidal volume (V_T) lung–thorax compliance (C) alveolar expansion index (I_a) and index for bronchiolar epithelial lesions (I_b) in premature rabbit neonates delivered on day 27 of gestation*

... lation with ... are given as $\bar{x} \pm$ S D

	Without PEEP (n=10)	With PEEP (n=10)	p
V_T (ml/kg)	12 62±8 78	24 87±5 88	<0 01
C (ml/cm H_2O kg)	0 36±0 25	0 69±0 16	0 01
I_a	0 42±0 11	0 66±0 35	NS
I_b	0 12±0 07	0 06±0 05	<0 05

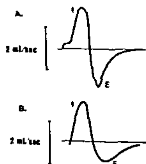


Fig 2 Recordings of air flow in two premature rabbit neonates after 10 min ventilation with standardized tidal volume (10 ml/kg) without PEEP (A) and with PEEP (5 cm H_2O) (B) Body weight in both fetuses was 31 g The horizontal line corresponds to zero flow (A) Inspira ... con ... al ex ... ar ac ... expiratory air flow is low I=inspiration E=expiration

Table 1. *Survey of the material*

Treatment	Number of neonates	Body weight (g, $\bar{x}$±S D)
1 Standardized V_T (10 ml/kg) without PEEP	12	33±4
2 Standardized V_T (10 ml/kg) with PEEP (5 cm H_2O)	12	31±4
3 Standardized P_I (35 cm H_2O) without PEEP	10	34±2
4 Standardized P_I (35 cm H_2O) with PEEP (5 cm H_2O)	10	34±5

was immediately opened, the vessels of the uterus were clamped with a large hemostat, and the fetuses removed after hysterotomy After birth the fetuses received intraperitoneal injections of 0 1 ml sodium pentobarbital (6 mg/ml) and 0 1 ml pancuronium bromide (Pavulon® 0 2 mg/ml, Organon, Holland) They were tracheotomized, kept at 37°C in a volume-constant body plethysmograph and connected to a Harvard Rodent Ventilator 680, set at a frequency of 60/min During the period of artificial ventilation, the fetuses were fixed in dorsal position by ECG electrodes through their paws Only fetuses showing ECG evidence of cardiac activity throughout the period of ventilation were included in the study

Ventilation

Insufflation pressure (P_I) and pressure changes in the plethysmograph were registered simultaneously by means of pressure transducers (EMT 33 and 34 Siemens-Elema, Stockholm, Sweden) and recorded on a Mingograph 81 (Siemens-Elema)

The fetuses were divided into four groups (Table 1)

1 Ventilation with standardized tidal volume (V_T= 10 ml/kg), without PEEP

2 Ventilation with standardized V_T (10 ml/kg) and PEEP (5 cm H_2O)

3 Ventilation with standardized P_I (35 cm H_2O, according to our previous experience this pressure level is usually required to maintain V_T in the range of 10 ml/kg in control fetuses at this day of gestation) (13) without PEEP

4 Ventilation with standardized P_I (35 cm H_2O) and PEEP (5 cm H_2O)

In groups 1 and 2, air flow was recorded with a differential pressure transducer (EMT 32C Siemens Elema) connected to the plethysmograph via a specially designed Fleisch tube (11)

All animals were ventilated with air Ventilation was limited to 10 min, as epithelial lesions develop in control fetuses within this period (14)

Histologic and morphometric techniques

After artificial ventilation, the fetuses were killed by intraperitoneal injection of Mebumal vet ® (0 5 ml) The chest of the fetus was opened and a cannula inserted into the pulmonary trunk The lungs were insufflated at a pressure of 35 cm H_2O The endotracheal pressure was then lowered to 15 cm H_2O, this pressure was maintained during the subsequent perfusion The pulmonary artery was perfused for 15 min with a mixture of 1% glutaraldehyde and 3 5% formaldehyde, at a pressure of 65 cm H_2O After perfusion, the lungs and the heart were left in situ and kept in 10% formaldehyde for at least 24 hours

Paraffin sections from the basal portions of the lungs were stained with hematoxylin and eosin and examined microscopically. The relative volumes of the alveolar spaces (V_a) and solid parenchyma (V_p, including interalveolar septa, vessels, bronchial and bronchiolar walls) were determined by point counting (3) The alveolar expansion index (I_a) was calculated by the formula

$$I_a = \frac{V_a}{V_p} \qquad (6)$$

The same sections were reexamined in high power fields (HPFs) and the number of HPFs showing evidence of bronchiolar epithelial necrosis were counted and related to the total number of HPFs examined according to the formula

$$I_b = \frac{\text{no of HPFs with lesion}}{\text{no of HPFs examined}} \times I_a \qquad (13)$$

The alveolar expansion index is used here as a correction factor, to compensate for variations in the volume of the alveolar compartment that might influence the spacing of the bronchioles The Wilcoxon two sample two tailed test and the Chi square test were used for statistical comparison

RESULTS

Comparison of results in groups 1 and 2 shows that the standardized V_T was maintained with a significantly lower pressure in fetuses treat-

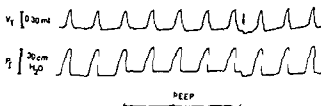


Fig 1 Recordings of tidal volume (V_T) and insufflation pressure (P_I) in premature rabbit neonate (body weight 31 g) during positive pressure ventilation with a short period of PEEP (5 cm H_2O), the setting of the ventilator pump is constant during this phase of the experiment During the PEEP period, V_T increases although the effective insufflation pressure decreases, reflecting increased compliance of the lung–thorax system (from 0 30 ml/cm H_2O kg to 0 35 ml/cm H_2O kg during PEEP) The baseline shift at the end of the PEEP period (arrow) corresponds to a reduction of FRC of approximately 0 04 ml

Table 2 *Insufflation pressure (P_I), lung–thorax compliance (C), alveolar expansion index (I_a) and index for bronchiolar epithelial lesions (I_b) in premature rabbit neonates, delivered on day 27 of gestation*

The observations were made after 10 min ventilation with standardized tidal volume (10 ml/kg) with or without PEEP (5 cm H_2O) Values are given as $\bar{X} \pm$S D

	Without PEEP (n=12)	With PEEP (n=12)	p<
P_I (cm H_2O)	34±6	22±4	0.002
C (ml/cm H_2O kg)	0.35±0.09	0.53±0.11	0.002
I_a	0.59±0.15	0.67±0.18	NS
I_b	0.16±0.14	0.04±0.07	0.02

Table 4. *Maximal inspiratory and expiratory air flow and inspiratory air flow pattern in premature rabbit neonates, delivered on day 27 of gestation*

The observations were made after 10 min ventilation with standardized tidal volume (10 ml/kg) with or without PEEP (5 cm H_2O) Values are given as $\bar{X} \pm$S D

	Without PEEP (n=12)	*With* PEEP (n=12)	p<
Maximal inspiratory air flow (ml/sec)	1.76±0.28	1.56±0.19	NS
Maximal expiratory air flow (ml/sec)	1.25±0.21	0.94±0.17	0.002
Biphasic inspiratory air flow pattern	9/12	0/12	0.001

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Table 3 *Tidal volume (V_T) lung–thorax compliance (C) alveolar expansion index (I_a) and index for bronchiolar epithelial lesions (I_b) in premature rabbit neonates delivered on day 27 of gestation*

The observations were made after 10 min ventilation with standardized insufflation pressure (35 cm H_2O) with or without PEEP (5 cm H_2O) Values are given as $\bar{X} \pm$S D

	Without PEEP (n=10)	With PEEP (n=10)	p
V_T (ml/kg)	12.62±8.78	24.87±5.88	<0.01
C (ml/cm H_2O kg)	0.36±0.25	0.69±0.16	0.01
I_a	0.42±0.11	0.66±0.35	NS
I_b	0.12±0.07	0.06±0.05	<0.05

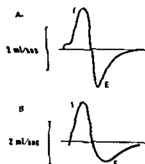


Fig 2 Recordings of air flow in two premature rabbit neonates after 10 min ventilation with standardized tidal

tory air flow is biphasic, with a brief period of low, constant flow preceeding accelerating air flow Maximal expiratory air flow is high (B) Air flow shows linear acceleration during early inspiration Maximal expiratory air flow is low I=inspiration E=expiration

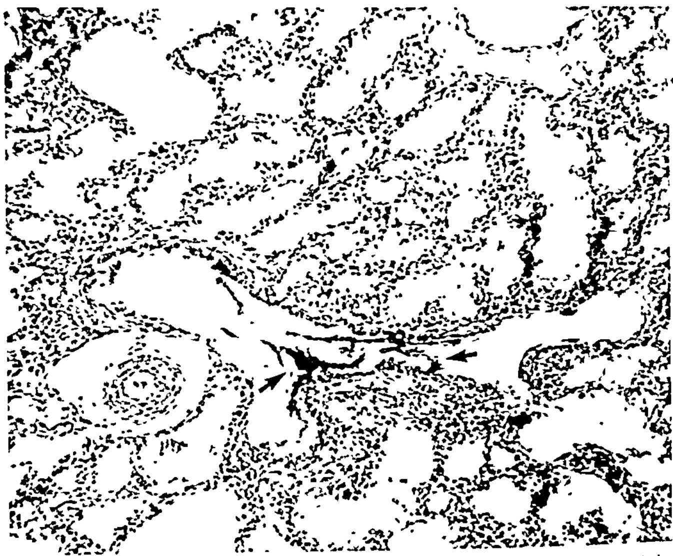


Fig 3 Detail of histologic lung section from premature rabbit neonate delivered on day 27 of gestation after 10 min of positive pressure ventilation without PEEP There is prominent necrosis and desquamation of bronchiolar epithelium (arrows) Hematoxylin and eosin ×175

acceleration throughout the early inspiration phase, these fetuses also had the highest values for lung–thorax compliance in the group (≥0 42 ml/cm H_2O kg) and the lowest index values for epithelial lesions (I_b=0–0 02) All fetuses ventilated with PEEP showed linear acceleration of flow during early inspiration (Fig 2)

Histologic examination of the lungs revealed large amounts of unresorbed fluid and poor alveolar air expansion in most fetuses, without apparent differences between the groups I_a was slightly higher in the groups of fetuses treated with PEEP, but the difference was not statistically significant Bronchiolar epithelial necrosis and desquamation of the epithelium (Fig 3) were found in all fetuses ventilated with standardized P_i Six of the fetuses ventilated with standardized V_T were free from epithelial lesions, five of these fetuses belonged to the group treated with PEEP Morphometric analysis showed epithelial lesions to be clearly reduced in the two groups ventilated with PEEP The difference in I_b between these groups and controls is statistically significant ($p<0$ 02 and <0 05 Tables 2 and 3)

DISCUSSION

Rabbit neonates removed on day 27 of gestation have a low content of surfactant phospholipids in their pulmonary fluid (7, 10) and

expansion of the lungs at birth is therefore hampered by high surface tension at the air-liquid interface Another well known consequence of surfactant deficiency is that any terminal airspaces that have become aerated with the first breaths tend to collapse during expiration, premature fetuses characteristically fail to establish functional residual capacity (FRC) during early neonatal ad iptation (11)

When, as in the present experiments, surfactant deficient rabbit neonates are ventilated with a pressure high enough to ensure adequate ventilation of the lungs the application of PEEP apparently leads to increased lung–thorax compliance and reduced expiratory flow These effects on lung mechanics are explained if PEEP prevents alveolar collapse at end expiration This would mean that an FRC is established (cf Fig 1) and that the inspiration phase is therefore shifted towards a more compliant range of the pressure-volume isotherm Similar observations have been made during artificial ventilation of premature lambs (20)

End expiratory alveolar collapse could thus explain the biphasic inspiratory flow pattern noted in most fetuses ventilated without PEEP The characteristic "hump of the tracing probably represents a period when flow is retarded by high surface forces (capillarity) in collapsed or fluid filled terminal conducting airways This resistance has to be overcome before the alveoli open and effective lung aeration takes place In animals treated with PEEP on the other hand, alveolar collapse does not occur and the acceleration of flow is therefore linear throughout the early inspiratory phase

Previously we have shown that surfactant deficiency is important in the pathogenesis of bronchiolar epithelial lesions and it has been suggested that the airway epithelium is disrupted secondarily to overdistension of the preterminal conducting airways (5, 8, 15, 17, 18, 19) We have also demonstrated that bronchiolar epithelial lesions can be prevented by tracheal instillation of natural surfactant l [illegible]

[illegible] tary surfactant In fetuses with surfactant deficiency, PELP presumably promotes uniform aeration of the alveolar compartment Thus, the air liquid interface remains at alveolar level throughout the ventilatory cycle, and resistance to aeration due to capillarity in finer conducting airways is avoided This results in linear acceleration of flow during early inspiration, and reduces the risk of overdistension of bronchioles (cf 18) Furthermore, since variations in airway dimension during ventilation depend on the expansion pattern of the surrounding parenchyma (9), application of PEEP may lower the risk of shear stress in the airway mucosa by favoring uniform aeration of the alveolar compartment It is noteworthy that the three animals ventilated *without* PEEP and yet showing a linear acceleration of flow during early inspiration, had comparatively high lung–thorax compliance and few epithelial lesions, suggesting a nearly mature surfactant system (cf 15)

Our results demonstrate that PEEP has a beneficial effect on lung mechanics during artificial ventilation of premature newborn rabbits, and that it reduces the risk of bronchiolar epithelial lesions, presumably by promoting uniform expansion of the pulmonary parenchyma

ACKNOWLEDGEMENTS

Financial support was provided by the Swedish Medical Research Council (project no 3351) the Swedish National Association against Heart and Chest Diseases and K I s Predoctoral Research Fund We thank M Cotler Fox for editorial assistance

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Submitted Dec 3 1979
Accepted April 3 1980

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Acta Pædiatr Scand 69 603–606, 1980

EFFECT OF POSITIVE-END-EXPIRATORY-PRESSURE ON RIGHT VENTRICULAR OUTPUT IN LAMBS WITH HYALINE MEMBRANE DISEASE

R B COTTON DANIEL P LINDSTROM KEITH S KANAREK, HÅKAN SUNDELL and MILDRED T STAHLMAN

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ABSTRACT Cotton R B, Lindstrom, D P, Kanarek, K S, Sundell, H and Stahlman, M T (Department of Pediatrics, Vanderbilt University Medical Center, Nashville, Tennessee, USA) Effect of positive-end-expiratory pressure on right ventricular output in lambs with hyaline membrane disease Acta Paediatr Scand, 69 603, 1980.—Right and left ventricular outputs, pulmonary and systemic blood flows, and blood flows in both directions through the ductus arteriosus were measured before and during positive-end expiratory pressure in 5 premature lambs with induced hyaline membrane disease During positive-end-expiratory pressure, right ventricular output increased in all lambs without any significant change in left ventricular output or pulmonary vascular resistance Left to-right ductus flow decreased in lambs which initially had large left to-right ductus shunts Significant right to-left ductus flow did not occur before or during positive-end-expiratory pressure

KEY WORDS Hyaline membrane disease, right ventricular output, positive-end-expiratory pressure

Infants with hyaline membrane disease (HMD) have complex patterns of extrapulmonary shunting through fetal circulatory pathways (6) Initially a right to left shunt at the foramen ovale predominates With time this pattern gradually changes so that beyond 36 hours into the disease there are frequently large left to right shunts through the ductus arteriosus Large right to left shunts at the ductus are uncommon at any time and usually occur only with systemic hypotension or very low arterial oxygen levels

Since the beneficial effect of positive end expiratory pressure (PEEP) in improving the oxygenation of infants with HMD was first documented in 1971 (4) this type of ventilatory assistance has become standard treatment However the effect of PEEP on the central circulation in the presence of HMD has not been well described It is possible that PEEP may have a beneficial effect on the large extrapulmonary shunts present in infants with HMD To investigate that possibility, separate ventricular outputs, pulmonary and systemic flows, and ductus shunts were measured before and after the institution of PEEP in lambs with induced HMD

METHODS

Mixed Western and Dorset premature lambs delivered by caesarean section between 132 and 135 days of timed gestation (term=147 days) were immediately resuscitated with endotracheal intubation and administration of 60 to 100 percent oxygen by intermittent positive pressure ventilation (IPPV) without positive end expiratory pressure (PEEP) In most cases the ewe had been made hypotensive with intramuscular pentolinium tartrate the day prior to delivery in order to increase the incidence of severe HMD As part of resuscitation sodium bicarbonate and 20% dextrose were given as necessary to correct acidosis and hypoglycemia. French 5 Argyle catheters were placed in the lower abdominal aorta through an umbilical artery in a leg vein in the right

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METHODS

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carotid artery (CA) and in the left ventricle (LV) through the right brachial artery French 8 Swan Ganz balloon catheters were placed in the right ventricle (RV) via the right brachiocephalic vein and in the pulmonary artery (PA) via the right internal jugular vein Fluoroscopy and pressure tracings were monitored in order to place the PA catheter above the pulmonary valve and proximal to the ductus arteriosus

The lambs were ventilated with a Harvard volume controlled animal respirator at a rate of 40–50 breaths per minute a peak inspiratory pressure of 28–36 cm H O and an inspiratory expiratory ratio of 1 1 Airway pressure was measured in the expiratory line by an aneroid manometer

Baseline measurements were made of systemic and pulmonary arterial pressures arterial pH and blood gases and the components of cardiac output—left ventricular output (LVO) right ventricular output (RVO) systemic flow (SF) pulmonary flow (PF) left to right ductus flow (DFL) and right-to-left ductus flow (DFR)—with the lamb receiving IPPV but no PEEP After the completion of baseline studies 5–8 cm H O PEEP was added by placing the respirator expiratory line under water and serial measurements of arterial P_{O_2} were made When PEEP effect had been demonstrated by an increasing arterial P_{O_2} baseline studies were repeated The time between baseline and follow up studies ranged from 25 to 45 min and during this interval the lamb received no additional treatment other than IPPV with PEEP and a constant FIO_2

Arterial pressures were measured with Statham P23b strain gauge transducers and recorded on paper using a Sanborn 350 polygraph Blood gases and pH were measured with an Instrumentation Laboratory Model 113 blood gas analyzer

The components of cardiac output were measured by a modification of the microsphere technique of Makowski et al (5) Right and left ventricular output determinations were made simultaneously by constant rate blood withdrawal from PA and CA during slow infusions of two preparations of microspheres each with a different radionuclide label into RV and LV respectively During blood withdrawal the lamb was transfused isovolumetrically into a leg vein to maintain a constant blood volume

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Table 2 *Central flows before and during PEEP*

Flow component	n	Before (mean ± S.E.M.)	During (mean ± S.E.M.)	t*	p
RVO (ml kg^{-1} min^{-1})	5	137±29	241±35	13.61	<0.001
LVO (ml kg^{-1} min^{-1})	5	346±87	368±74	0.36	NS
PF (ml kg^{-1} min^{-1})	5	302±93	362±76	2.56	<0.1
SF (ml kg^{-1} min^{-1})	5	181±28	247±48	1.09	NS
DFL (% LVO)	3[b]	58±11	42±10	8.88	<0.025

* Paired t statistic.
[b] Values given for the 3 lambs with DFL ≥40% of LVO before PEEP. DFL in the other 2 lambs was 0 and 14% of LVO.

increased in 4 of the 5 lambs, there was no significant change in estimated pulmonary vascular resistance (50.5±18.8 vs. 50.1±16.0 mmHg ml^{-1} min^{-1}).

DISCUSSION

Co-existing right-to-left shunting through the foramen ovale and left-to-right shunting through the ductus arteriosus is common in infants with hyaline membrane disease (6). One result of this bi-directional shunting is an inequality in right and left ventricular outputs. Either a right-to-left foramen shunt or a left-to-right ductus shunt results in a greater proportion of combined cardiac output being handled by the left ventricle than by the right ventricle.

The previously described (2) inequality between ventricular outputs was also demonstrated in these premature lambs with hyaline membrane disease before PEEP was added to their ventilatory management. Part of the increased left ventricular output before PEEP is accounted for by a large left-to-right ductus shunt which was documented by the microsphere techniques in 3 of the 5 lambs. In addition, the presence of any right-to-left foramen shunting would have further contributed to increased left ventricular output.

All five lambs had a substantial increase in RVO following the application of PEEP, and as a group, this finding was highly significant. Concomitantly, there was only a small and statistically insignificant increase in LVO, so that with PEEP, the difference between right and left ventricular outputs was minimized.

The improved balance between right and left ventricular outputs following PEEP might have been achieved as a result of either a diminished left-to-right ductus shunt or a diminished right-to-left foramen shunt, or both. A decrease in left-to-right ductus shunting following PEEP was documented in the 3 lambs who had large ductus shunts before PEEP, contributing to the overall 36% improvement in systemic flow, from 181 to 247 ml kg^{-1} min^{-1}. Right ventricular output at the same time increased 76%, from 137 to 241 ml kg^{-1} min^{-1}. This increase in RVO is out of proportion to the increase in SF and cannot be accounted for solely on the basis of improved systemic venous return.

The disproportionate increase in RVO is consistent with the hypothesis that right-to-left foramen shunting decreased with PEEP. With decreased foramen shunting, a greater proportion of systemic venous return would contribute to right ventricular output rather than be diverted to LVO via the foramen, resulting in an improved balance between left and right ventricular outputs. As a corollary to this hypothesis, it is arguable that PEEP need have no direct effect on pulmonary vascular resistance under these conditions, and that left-to-right ductus flow is diverted forward into the systemic circulation by the increased RVO.

Why PEEP should decrease right-to-left foramen shunting is not readily apparent. This

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The lambs were ventilated with a Harvard volume controlled animal respirator at a rate of 40–50 breaths per minute, a peak inspiratory pressure of 28–36 cm H_2O, and an inspiratory expiratory ratio of 1 1 Airway pressure was measured in the expiratory line by an aneroid manometer

Baseline measurements were made of systemic and pulmonary arterial pressures, arterial pH and blood gases, and the components of cardiac output—left ventricular output (LVO), right ventricular output (RVO), systemic flow (SF), pulmonary flow (PF), left to right ductus flow (DFL) and right-to-left ductus flow (DFR)—with the lamb receiving IPPV, but no PEEP After the completion of baseline studies, 5–8 cm H_2O PEEP was added by placing the respirator expiratory line under water and serial measurements of arterial P_{O_2} were made When PEEP effect had been demonstrated by an increasing arterial P_{O_2} baseline studies were repeated The time between baseline and follow up studies ranged from 25 to 45 min and during this interval the lamb received no additional treatment other than IPPV with PEEP and a constant FIO_2

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Acta Pædiatr Scand 69 607–611, 1980

CHRONIC RENAL FAILURE IN SWEDISH CHILDREN

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ABSTRACT Helin, I and Winberg, J (Departments of Paediatrics, Malmo General Hospital, Malmo, University of Lund, and Karolinska Hospital, Karolinska Institute, Stockholm, Sweden) *Chronic renal failure in Swedish Children* Acta Paediatr Scand, 69 607, 1980.— In a retrospective study covering the years 1974 to 1977 the prevalence of non-terminal renal failure in Swedish children on to 15 years of age was registered as 4 50 per million total population The mean yearly incidence of terminal renal failure during the same period was registered as 0 94 per million total population Nephronophthisis was the most common single cause of renal failure No case of coarse renal scarring due to recurrent urinary tract infections was reported The Swedish study shows a good agreement with an earlier *Swiss one concerning the diseases causing chronic renal failure* in children The frequency of chronic renal failure is expected to be essentially unchanged until breakthroughs occur in research on glomerulonephritis

KEY WORDS Chronic renal failure, glomerulonephritis *nephronophthisis, hereditary* nephropathies

Since 1970 there has been an increasing use of haemodialysis and renal transplantation in children with terminal renal failure The possibilities to improve the condition by dietary treatment as well as by other supportive measures have also obtained increased attention as have the many psychological social, and economic consequences associated with chronic renal failure (CRF) and its active treatment

An active approach necessitates an analysis of the size of the problem This study examines the incidence of terminal renal failure and prevalence of *non terminal* renal failure in Swedish children In addition the causes of CRF are analysed

PATIENTS AND METHODS

Demography and organisation of care for renal disease

[illegible] special ized unit for paediatric nephrology Paediatric nephrologists are working in a few of the larger paediatric units

Children with non terminal renal failure are taken care of at local paediatric departments often in close collaboration with the paediatric nephrologists whereas those who are dialysed and/or transplanted are cared for at one of the nephrological centres for adults

Procedure All the 44 paediatric departments and the five departments for paediatric surgery as well as the nine nephrological centres have participated in this study They have answered a questionnaire concerning the number of children who during the years 1974–1977 were treated or checked for chronic renal failure The study concerns all children between one and 15 years of age Infants below the age of one year are not included Information concerning probable diagnosis and the course of the disease has also been collected In four patients the diagnosis was uncertain Their original records have been reviewed and a best guess diagnosis was made

Definitions Chronic renal insufficiency has been defined as a persistent elevation of serum creatinine to more than 180 μmol/l during a period longer than two months The children have been divided into two groups terminal and non terminal renal failure Terminal renal failure was defined as occurring when disease resulted in dialysis renal transplantation or death

Prevalence of non terminal renal failure is defined as the number of sick children at the start of the period in addition to the number of children becoming ill during

Published in honour of professor Andrea Prader Zurich at his 60th birthday

effect did not appear to result from a decrease in pulmonary vascular resistance In addition, there was a decrease rather than an increase in left-to right ductus flow following the application of PEEP

Pressure differences between the left atrium and inferior vena cava are small and difficult to interpret in HMD because of distortion by intrathoracic pressure changes with ventilation In human infants, end expiratory a and v wave pressures are higher in the left atrium than the inferior vena cava, favoring closure of the foramen ovale (6) It is likely that right-to-left foramen shunting occurs during the marked negative intrathoracic pressure produced by an inspiratory effort against a poor pulmonary compliance Although the lambs in this experiment were mechanically ventilated, they made vigorous inspiratory efforts between the cycles of intermittent positive pressure The addition of PEEP may have reduced a pressure differential that promoted right to left shunting between the inferior vena cava and left atrium during inspiration

The possibility that right-to left shunting at the foramen ovale is reduced by PEEP is consistent with the findings of Corbet et al (1) who showed that human infants with HMD treated with PEEP have a decrease in total venous admixture that is almost totally accounted for by a reduction in true right-to left shunt Although they interpreted their findings as improved ventilation in a lung compartment which is open but severely underventilated, a reduction in right to left foramen shunt would have led to the same results In a study of immature goats with unspecified lung disease, Egan & Hessler (3) noted that PEEP was accompanied by a decrease in foramen ovale shunt in 4 out of 9 studies of a heterogenous group of animals

The results of this study indicate that there is a substantial and significant increase in RVO when HMD lambs are treated with PEEP, and that the imbalance between right and left ventricular outputs is reduced These findings are consistent with the hypothesis that one favorable effect of PEEP in HMD is decreased foramen shunting, increased RVO, and diversion of left-to right ductus flow forward into the systemic circulation

ACKNOWLEDGEMENTS

The authors would like to acknowledge the valuable technical assistance of Mr David Oliver and Mr Rao Gaddipatti as well as the enthusiastic cooperative effort of Drs Zvi Friedman Fredrik Serenius and Marilyn Escobedo without whose help these experiments would not have been successful Also we thank Dr Fred Battaglia for drawing our attention to the value of microspheres in the quantitation of flows in the presence of complex shunts

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Table 3 *Prevalence of non terminal renal failure (NTRF) and incidence of terminal renal failure (TRF) in Swedish children as compared to result from other studies*

	Prevalence of NTRF per mill total population	Mean yearly incidence of TRF per mill total population
Present study	4 5	0 94
Switzerland[a]	4 5	3 36
Germany (Western part)[b]	3 9	1 13
Germany (excl Bayern)[c]	2 5	
Denmark[d]		2 8

[a] Age 6 months–16 years (3)
[b] Age 3 months–15 years (5)
[c] Age 0–16 years (5)
[d] Age 0–15 years Information from death certificates (2)

dren (21%), and was thus the leading single cause of renal failure in this study Fifteen had terminal failure, three of whom died

Focal or asymmetric parenchymal defects is a term covering any cause of focal parenchymal reduction, whether congenital or acquired such as infection circulatory accident congenital dysplasia, and hypoplasia Nine children (12%) belonged to this group, including two patients with congenital cystic disease of the kidneys None underwent a kidney biopsy Three children in this group had terminal renal failure, two of them died, both with bilateral renal hypoplasia

Primary metabolic diseases hereditary (?) nephropathies haemolytic uraemic syndrome and Wilms tumor were associated with chronic renal failure in eight patients (10%) Renal biopsies failed in the two patients with suspected hereditary nephropathy

DISCUSSION

There are few reports on the prevalence and incidence of CRF in children as well as in adults Studies based upon information from death certificates (1, 3) are often inaccurate They will only give a crude estimate of the frequency and international comparisons may be difficult In addition they give no information on suitabilities of dialysis or transplantation

The prevalence of non-terminal renal failure (NTRF) is consistent with that reported from Switzerland (6), 4 50 per million total population Other studies have shown a lower prevalence, cf Table 3 Incomplete reporting is the most probable explanation for the differences shown in Table 3, although confounding factors such as differences in the proportion of children in the total population or local occurrence of a specific disease cannot be ruled out (2)

The mean yearly incidence of terminal renal failure (TRF) among the Swedish children is considerably lower than that in Switzerland and in the western part of Germany, cf. Table 3 The reason is not obvious A possible explanation would be the fact that these studies include children from 6 and 3 months of age, respectively However, in the Swiss series only 4 children became ill between 6 and 12 months of age (Leumann, personal communication 1979) Since the prevalence of NTRF was the same in this and the Swiss studies a guess would be, that the difference is real This will, however, pend further analysis With regard to the German study (7) the possibility still exists that differences might be explained by the fact that in this study children were included from the age of 3 months, whereas ours started from 12 months onwards As the Danish study (1) was based solely upon information from death certificates it is not adequately comparable

In Table 4 the different groups of renal diseases in the Swedish children are compared with those of the Swiss series Congenital anomalies are more common as a course of TRF in Switzerland In the Swiss series two children below the age of 12 months had TRF due to congenital anomalies (Leumann, personal communication 1979) Excluding them will reduce the percentage only marginally TRF

39 80 875

Table 1 *77 children with chronic renal failure, main categories (%)*

Obstruction (congenital or acquired)	30
Glomerulonephritides	27
Nephronophthisis	21
Focal or asymmetric parenchymal defect	12
Miscellaneous	10
	100

the period studied and related to the mean total population

Incidence of terminal renal failure is defined as the number of new cases per annum related to the mean total population

RESULTS

All the 58 consulted clinics responded to the questionnaire indicating that the survey includes all Swedish children, one to 15 years of age, who were examined or actively treated through the years 1974 to 1977 for chronic renal failure Altogether 77 children were reported Thirty-six of the patients had non terminal renal failure on the 31st of December 1977 This means a period prevalence of non-terminal renal failure of 4 50 children per million total population equal to 20 0 per million children, one to 15 years of age At the beginning of 1974 eleven had terminal failure and another 30 progressed to this stage by the end of 1977 (1974 6 1975 4, 1976 9 1977 11) This means a yearly incidence of terminal renal failure corresponding to 0 94 per million total population and 4 17 per million children one to 15 years of age

The patients have been grouped according to main categories (Table 1) as well as to the basic cause of the renal failure (Table 2)

Obstructive anomalies were found in 23 children (30%) During the period studied four progressed to terminal renal failure two of whom died

Glomerulonephritides of different kinds were found in 21 instances (27%) Fourteen of these patients had terminal renal failure and eight died during 1974 to 1977 Renal biopsy was performed in 14 patients, i e in all patients with proliferative glomerulonephritides in the patient with an IgA nephritis in five of the six patients with nephrotic syndrome and in two of the three patients with Schoenlein-Henoch nephritis In the children categorized as having proliferative glomerulonephritides the damage was in most instances so severe that a more precise classification was difficult In the nephrotic syndrome patients the following histological classifications were made proliferative glomerulonephritis (1) end stage chronic glomerulonephritis (1) congenital nephrosis (1 not Finnish type) and focal, segmental glomerulosclerosis (2)

Nephronophthisis was present in 16 chil

Table 2 *Causes of CRF*

	No		Terminal failures		Dead
Obstructive anomalies		23		4	2
Urethral valves/stenoses	16		2		
Urethral stenosis	5		1		
Neurogenic bladder disease	2		1		
Glomerulonephritides		21		16	8
Proliferative glomerulonephritides	6[a]		5		
IgA nephritis	1[a]		1		
Nephrotic syndrome	6[b]		4		
Schoenlein Henoch nephritis	3[b]		2		
Lupus nephritis	2		1		
Hereditary nephritis (Alport s syndrome)	3		1		
Focal or asymmetric parenchymal defects		9		3	2
Hypoplasia/dysplasia/aplasia	7		3		
Cystic disease	2		–		
Nephronophthisis		16		15	3
Metabolic disorders		3		2	1
Cystinosis	2		2		
Laurence Moon Biedl s syndrome	1		–		
Miscellaneous		5		1	
Wilms tumor	1		1		
Hereditary nephropathy (?)	2		–		
Haemolytic uraemic syndrome	2		2		
		77		41	16

[a] Renal biopsy in all [b] Renal biopsy in 5/6 and 2/3 respectively

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Submitted Jan 18 1980
Accepted Febr 20 1980

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Table 4 *Comparison of chronic non-terminal renal failure (NTRF) and terminal renal failure (TRF) in Sweden and Switzerland*

The Swedish study includes children one to 15 years of age the Swiss study those between six months and 15 years

Diagnosis	NTRF		TRF	
	Sweden n=36	*Switzerland n=28*	*Sweden n=41*	*Switzerland n=68*
Acquired nephropathies	19 4	14 3	39 0	55 9
Hereditary nephropathies including nephronophthisis	11 1	3 6	43 9	11 8
Cystic disease	5 6	14 3	–	1 5
Hypoplasia	11 1	14 3	7 3	4 4
Other congenital anomalies	52 8	53 5	9 8	26 4

after the age of 12 months is still considerably higher in Switzerland than in Sweden A distinct difference is also noted in the occurrence of hereditary nephropathies Whether this is due to differences in gene frequency or in definitions is uncertain

The low number of children with CRF caused by low obstruction might be explained by the fact that infants and small children with their first urinary tract infection will usually have an early intravenous pyelography Because of this obstructions are demonstrated and operated upon early The pioneering work performed in the early 50 s by Kjellberg, Ericsson & Rudhe (5) might have contributed to the results

Nephronophthisis was the most common single cause of CRF in this study (Table 2) This disease appears not only in a recessive but also in a dominant form (8) Genetic counselling can decrease this frequency only marginally

Acute pyelonephritis without anatomical obstruction is the most common renal disease in children In spite of this, not one single patient with coarse renal scarring due to recurrent urinary tract infections was reported to have CRF during the four years covered by this study The explanation might be that acute pyelonephritis only rarely causes renal insufficiency during childhood It is also possible that the alert diagnosis of neonatal and early infantile pyelonephritis together with a close survey of children with recurrent urinary tract infections during the last two decades have influenced the frequency (9) Probably a careful follow-up of infants and children with urinary tract infections will even decrease the incidence of CRF in adults as well as of hypertension and complications during pregnancy in the future It has been known for almost three decades (4), that recurrent urinary tract infection during childhood in many instances is the forerunner of these problems

The figures presented in this study are minimum figures They may, however, provide a basis for evaluation of our future resources and requirements in dealing with uraemic children The frequency of CRF in children is expected to be essentially unchanged until breakthroughs occur in research on glomerulonephritis

ACKNOWLEDGEMENTS

This work was supported by The Swedish Medical Research Council (grant no 19X 765) and Greta and Johan Kock s Foundation for Medical Research

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Acta Pædiatr Scand 69 613–617, 1980

CHANGES IN BLOOD PRESSURE DURING THE FIRST YEAR OF LIFE

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ABSTRACT Uhari, M (Department of Paediatrics, University of Oulu, Oulu, Finland) Changes in blood pressure during the first year of life Acta Paediatr Scand, 69 613, 1980 —Changes in blood pressure during the first year of life were investigated in a one-year follow up study The blood pressures were measured at the age of one day from 245 newborns, at the age of four or five days from 224 infants, at the age of four months from 105 infants, and at the age of one year from 68 infants Blood pressure increased considerably during the first five days The increase of mean systolic blood pressure was markedly bigger, from 59 to 90 mmHg, than that of mean diastolic blood pressure, which was from 43 to 55 mmHg The correlations of blood pressure were calculated with prenatal history, physical measurements and parental blood pressure Significant correlations were found with physical factors but not with the prenatal history or parental blood pressure The tracking of the blood pressure was not good The distribution of the blood pressure values was normal It is concluded that for the time being there are no reliable predictive factors to hypertension which could be found in early infancy

KEY WORDS Blood pressure infancy, inheritance, tracking

Recently it has been accepted that hypertension has its roots in childhood (9 10) The possible factors predicting the subsequent development of hypertension have been extensively studied (3, 5 22) Familial aggregation and tracking of blood pressure (BP) are phenomena already found in school children (3 5, 16 22 23) The BP of newborns and infants has been investigated from the methodological point of view while reports concerning determinants of BP during this period of life are few (4 6 17 19) Using the Doppler method, this study investigates changes in BP by following a selective group of children during the first year of life The correlations with prenatal history maternal and paternal BP are calculated

SUBJECTS AND METHODS

All the 245 healthy newborns born at the University Central Hospital of Oulu during May 1978 were included in the study The information concerning the mothers BP proteinuria oedema and weight gain during pregnancy was collected retrospectively from the prenatal records

The BP on the first day was recorded between four and 22 hours of life The second BP measurement was performed on the fourth day from 91 newborns and on the fifth day from 133 All the measurements were performed in the morning after feeding Twenty one children left the hospital earlier or became ill and thus were not included in the follow up After four months an invitation to 143 mothers living near the hospital was sent for a further visit to the clinic One hundred and five mothers brought their babies to the third measurement From these 99 were willing to attend a further follow up and were invited to the fourth measurement At this occasion 73 one year-old children visited the clinic Five of these infants were so restless that the BP could not be measured The participation rate was 73 and 74% of invitations at the third and fourth measurements

During the measurements children were awake and not crying At the first three measurements they were in the supine position while at the age of one year BP was measured when the child was sitting in the mother s arms The arm of the infant was resting on the arm of the mother

The BP measurements were performed with an ultrasound device (Arteriosonde® 1020 Roche) which has been found to be suitable for scientific purposes (17) Calibration was performed by a mercury manometer Aquasonic an ultrasound transmission gel was applied to the transducer, the cuff was placed firmly around the upper right arm and the transducer over the brachial artery the cuff was inflated to approximately 150 mmHg and released at a rate of 2–3 mmHg per second The cuff sizes were 4×10 cm (first two measurements) 5 5×10 cm (at 4

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Table 2 *The correlation coefficients between the blood pressure and physical factors at respective ages*

Age	BP	Physical factor Weight	Height	Body surface area	Upper arm circumference
1 day	Systolic	0 15*	0 14*	0 16*	–
	Diastolic	0 13*	0 09	0 14*	–
4–5 days	Systolic	0 18**	–	0 20***	–
	Diastolic	0 14*	–	0 14*	–
4 months	Systolic	0 47***	0 30**	0 23*	–
	Diastolic	0 32***	0 27**	0 20*	–
1 year	Systolic	0 43***	0 24*	–	0 34**
	Diastolic	0 46***	0 29*	–	0 43***

* $p<0\ 05$ ** $p<0\ 01$ *** $p<0\ 001$

of four or five days still had their BP over this level at the age of four months Three children out of 10 kept their high tracks between the ages of four months and one year Two children out of 14 kept their low values between the ages of four months and one year and thus the stability of the low values was no better than that of the high

The parental BP values were similar to those of Finnish adults Men had significantly ($p<0\ 001$) higher BP values than women Thirty mothers had a BP of 140/90 mmHg or more at the 20th week of pregnancy These mothers were classified as having hypertension The BP of 53 mothers increased to equal or exceeded this value later during the pregnancy and they were classified as having pre eclampsia The mothers BP during the pregnancy had no correlation with the BP of the newborns The BP values of the three groups of children whose mothers were normotensive, hypertensive or having pre eclampsia did not differ significantly The BP of the mother after delivery did not correlate significantly with that of the newborn The mode of the delivery did not cause any difference in the BP of the newborns

Thirty four mothers smoked during the pregnancy The BP of the newborns of these mothers was similar to that of the others Smoking habits of the mother during pregnancy correlated with the decreased weight of the newborn but not to the BP of the child even after excluding statistically the effect of the weight

No significant correlation was found between the BP of the fathers and that of the newborn and infants Neither did the prevalence of cardiovascular diseases in relatives correlate with the BP of the children The

Table 3 *The tracking phenomenon as calculated with the correlation coefficients between blood pressure values measured at different age*

Age at the measurement	n	Systolic BP coefficient	p	Diastolic BP coefficient	p
1 to 4–5 days	224	0 24	<0 001	0 25	<0 001
4–5 days to 4 months	105	0 16	NS	0 26	<0 01
4 months to 1 year	68	0 17	NS	0 06	NS

NS=not significant

Table 1 *The mean BP values and standard deviations at different ages during the one-year follow up (mmHg)*

Age	Number of children	Systolic BP (S D)	Diastolic BP (S D)
1 day	245	59 (8)	43 (8)
4 days	91	71 (10)	49 (8)
5 days	133	74 (9)	51 (9)
4 months	105	86 (9)	50 (7)
1 year	68	90 (10)	55 (7)

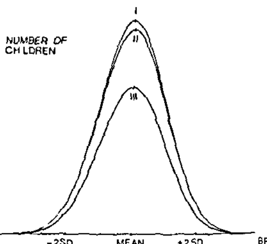


Fig 2 The distribution curves of systolic blood pressure at the age of one day (I $n=245$) four months (II n 105) and one year (III $n=68$) As tested statistically curves II and III were comparable to that of the normal distribution

months) and 6×15 5 cm (at age 1 year) The final value was the mean of three readings

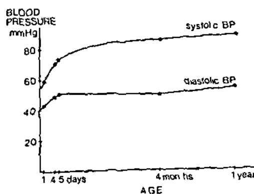
The BP of the mother was measured the day after the delivery with the method recommended by Rose & Blackburn (15) For diastolic BP the both Korotkoff fourth and fifth phases were recorded The BP of 196 fathers was measured as they visited the ward to see the mother and child All the BP measurements of the newborns and mothers were performed by the author and those of the fathers by the nurses on duty

The prevalence of cardiovascular diseases in close relatives was investigated by a questionnaire which was sent to the mother before the visit for the third BP measurement It was returned and checked on this visit

The normal distribution of the BP values was tested with the X^2 test (13) The curves were drawn by a computer The Pearson correlation coefficients were computed for the newborn infant BP Canonical variables were calculated between the BP of the children and that of the parents The significance of the variables was tested with the X^2 test and the t test Discrimination analysis was performed on the BP values at four months of age

RESULTS

The systolic and diastolic BP values of the children at different ages are presented in Table 1 The largest increase of BP occurred during the first five days of life (Fig 1) In these results the values at the age of four or five days are separated but in the further calculations this data are combined Boys had a tendency to a higher BP than girls but the difference was not statistically significant



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At the age of one day, the kurtosis of the distribution was too excessive and at the other ages the distributions were comparable to that of the normal distribution ($p<0.01$) (Fig 2) None of the distributions were skewed

The correlation coefficients were calculated between BP and physical parameters (Table 2) The upper arm circumference was measured only at the age of one year All the calculated correlation coefficients except between the height and the diastolic BP of the first day were statistically significant as tested with the t test (Table 2)

The tendency of the BP always to differ from the mean by a constant amount the so called tracking phenomenon was tested as a correlation of BP with the earlier measurement (Table 3) The stability of the values which were over one standard deviation above the mean was calculated separately Three children out of 19 who had their BP over one standard deviation above the mean at the age

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Submitted Oct 26 1979
Accepted March 3 1980

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DISCUSSION

The comparison of BP values of different studies should be done cautiously because of the many possibilities of measurement artefacts (12) On comparing the results of the one-year-old with those of Hennekens and co workers, significantly lower values were found particularly in the systolic BP (5) All children in Finland are given precise orders for a diet which contains little sodium and for avoiding obesity in infancy The recommended low sodium diet is well accepted by mothers Almost all babies regularly visit baby welfare centers These may be some of the reasons for the low BP values in this series

The distribution of BP in adults has been found to be skewed (14) It has been suggested that this is caused by the multigenetical inheritance of hypertension (14) The distributions in the present series during the first year of life were not skewed It can be speculated that the influence of the genetical factors causing hypertension is not evident until adulthood These speculations are supported by the finding that the skewness increases with the age of the population (20 21)

The size of the cuff plays an important role when measuring BP with the ultrasound method (11) Because the same size of cuff was used for every child at the same age it is possible that the positive correlation with the physical parameters obtained are artefacts caused by the indirect method used

Age seems to be one of the most important factors associated with changes of BP Especially, the velocity at which BP will change is associated with age In adults Smirk has shown that the rise with age of the BP was greater if the first degree relatives has hypertension as compared to the controls (18)

The tracking of BP and the inheritance of the BP level have been considered as possible parameters in order to find those who will get hypertension in adulthood (3, 5, 8, 19, 23) In children under the age of one year there were no indications of the inheritance of the BP level when correlating with the BP of the parents Again, the tracking phenomenon of BP was weak or totally lacking as in the follow-up one year later In previous studies it has been observed that the tracking phenomenon strengthens with age, similarly to the familial aggregation of the BP level (3 5, 8, 16) More important than the tracking of the BP as such would be the stability of the high BP values (2) In the present study the stability of neither the high nor the low values during the first year of life proved to be good

On the basis of these results it can be concluded that newborn period and infancy is a time for extensive BP changes Until now there is no reliable predictive factors of hypertension to be found in infancy Familial aggregation and tracking of BP can sometimes be found already in infancy but these phenomena do not occur constantly and are so weak that the group at risk for hypertension cannot be reliably found

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Submitted Oct 26 1979
Accepted March 3 1980

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Acta Pædiatr Scand 69 619–624, 1980

SOME EPIDEMIOLOGICAL ASPECTS OF CONGENITAL HEART DISEASE IN DENMARK

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ABSTRACT Laursen, H Bækgaard (Departments of Cardiology and Paediatrics, University Hospital Århus, Denmark) Some epidemiological aspects of congenital heart disease in Denmark Acta Paediatr Scand, 69 619, 1980.—Information on 5249 children with congenital heart disease (CHD) in the age group 0–15 years was collected from all paediatric and [illegible] Denmark and furthermore, from death certificates The mean [illegible] female, [illegible] cardiac [illegible] by pers [illegible] 7 0% [illegible] all 5249 children was 25% at 1 month, 42% after 5 years, and [illegible] CHD was found in 135 cases among 5835 siblings (2 3%)

KEY WORDS Congenital heart disease, epidemiology, prevalence, lethality, genetics

The first attempt to estimate the prevalence and relative frequency of congenital heart disease (CHD) in a rather large population was made by MacMahon et al (13) in 1953 During the following years several similar studies were published (1 2 3 6 10 14 15 18) A country wide epidemiological study covering a period of 11 years has not yet been published but would probably be valuable because case selection would be avoided and incorrect classification of single cases of CHD would be of minor importance on account of the large number of cases involved The present paper is based upon a thesis on CHD in Denmark (12) and includes some major epidemiological aspects of CHD in comparison with the results of other authors

MATERIALS AND METHODS

The study is limited to the period from Jan 1 1963 to Dec 31 1973 and includes all children in the age group 0–15 years in whom the initial CHD diagnosis was made during this period in Denmark Children from Greenland and the Faroe Islands were not included The results are based on information from hospital records in all paediatric and cardiological departments in Denmark and in a few cases on death certificates only In classifying CHD the book *Classification of Heart Disease in Childhood* (8) was used In the cases with multiple cardiac defects the major defect was the one considered most serious from a haemodynamic point of view

RESULTS

The study includes 5249 children In 30% of the cases, the cardiac diagnoses were established by clinical examination alone (X ray, ECG, auscultation), in 47% by heart catheterization, and in 23% by autopsy without previous heart catheterization

The prevalence is calculated as the number of children with CHD per 1000 children at risk The population at risk is calculated by exposing a cohort at birth to the actuarially age determined death rates existing for the whole of Denmark during the years in ques-

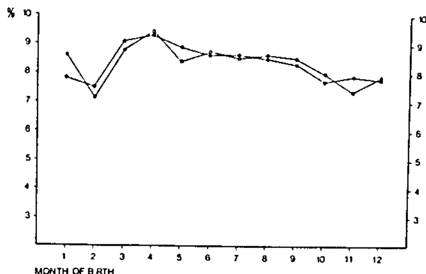


Fig 1 Month of birth of 5 249 child with CHD compared with that of non children O—O Month of birth of child with CHD ●—● month of birth children in general according to the offi statistics of Denmark

tion The prevalence is shown in Table 1 The lowest figure (5‰) is observed in 1963 and the highest during the years 1972–73 (7‰) The mean prevalence was 6 1‰ and roughly the same in different geographical parts of the country The highest prevalence was found on the island of Funen in 1972 (8 2‰) The age at first admission to hospital with a diagnosis of CHD was noted for each patient and used in the elaboration of cumulative detection tables The mean age at detection for all 5 249 cases was 25 86 months, 36% were diagnosed within the first month and 63% within the first year of life The sex distribution was males 52%, females, 48% Fig 1 shows the months of birth No statistical difference was observed in considering births per quarter year with expected values from all liveborn children in Denmark No sex difference was found in the time of birth of children with CHD, and the time of birth of children with individual cardiac malformations did not differ

Table 2 shows the relative frequency of cardiac defects The 491 children with no definite diagnosis had clinical signs of CHD (at least 2 of the following 3 clinical features were present (1) a systolic murmur > grade 3 or a diastolic murmur, (2) the ECG showed right hypertrophy in older children or left hypertrophy, (3) enlargement of the heart in X-ray) Of these 491 children 231 died without subsequent autopsy, the rest had mainly less severe cardiac defects and awaited heart ca eterization The 160 cases termed ot malformations" included such defects as v cular ring, anomalies of the coronary arteri double outlet right ventricle, primary p monary hypertension, aorto pulmonary w dow, anomalous pulmonary arteries and id pathic pulmonary dilatation The largest gro was ventricular septal defect (24%) and 43 of these patients were diagnosed with only clinical examination (the minimum crite were a grade 4 high frequency holosysto murmur heard at the left lower part of t sternum) Co existing cardio vascular m formations were found in 1 368 of 3 640 cas

Table 1 *Prevalence of CHD in Denma 1963–73*

Year	No of children with CHD	Prevalence (no per 1 000 children at risk)
1963	376	5 0
1964	418	5 4
1965	472	6 0
1966	478	5 9
1967	497	6 0
1968	467	5 7
1969	477	6 2
1970	506	6 8
1971	503	6 8
1972	524	7 0
1973	531	7 0
Total	5 249	6 1

Table 2 *Frequency of individual congenital cardio-vascular malformations based on 5 249 cases diagnosed in Denmark 1963–73*

Main diagnosis	1963–68 No	1969–73 No	1969–73 No	1969–73 %	Prevalence per 1 000 at risk
No diagnosis	265	226	491	9 4	0 58
Coarctation of the aorta	206	162	368	7 0	0 43
Aortic stenosis	116	129	245	4 7	0 29
Atrial septal defect[a]	256	238	494	9 4	0 58
Persistent ductus arteriosus	337	323	660	12 6	0 77
Common atrioventricular canal	74	60	134	2 6	0 15
Fibroelastosis + cardiomyopathia	81	41	122	2 3	0 14
Hypoplastic left heart	68	91	159	3 0	0 18
Pulmonary atresia with intact ventricular septum	20	13	33	0 6	0 04
Pulmonary stenosis[b]	159	153	312	5 9	0 36
Anomalous pulmonary venous drainage[c]	18	23	41	0 8	0 05
Single ventricle	46	31	77	1 5	0 09
Transposition of great arteries	145	108	253	4 8	0 29
Tricuspid atresia	15	31	46	0 9	0 05
Truncus arteriosus	41	34	75	1 4	0 09
Ventricular septal defect	598	661	1 259	24 0	1 48
Tetralogy of Fallot	162	142	304	5 8	0 36
Ebstein s anomaly	11	5	16	0 3	0 02
Other malformations	90	70	160	3 0	0 19
Total	2 708	2 541	5 249	100 0	6 14

[a] Ostium primum defect included
[b] Idiopathic pulmonary dilatation not included
[c] The partial form also included

Table 3 *Cumulative percentage lethality and mortality rate per 1 000 children at risk*

Main diagnosis	Cumulative percentage lethality at 5 years 1963–68	1969–73	Mantel Haensel test (9) χ^2	Total dead 1963–73	Rate/1 000 at risk
No diagnosis	73	27	26 00[a]	231	0 28
Coarctation of the aorta	73	63	1 39	207	0 24
Aortic stenosis	44	21	4 88[a]	48	0 06
Atrial septal defect	57	27	32 33[a]	147	0 17
Persistent ductus arteriosus	50	29	19 58[a]	204	0 24
Common atrioventricular canal	78	59	2 93	91	0 10
Fibroelastosis + cardiomyopathia	75	71	0 08	84	0 10
Hypoplastic left heart	100	100	2 53	159	0 18
Pulmonary atresia with intact ventricular septum	100	100	0 01	32	0 04
Pulmonary stenosis	15	6	3 64	22	0 03
Anomalous pulmonary venous drainage	63	57	0 00	21	0 03
Single ventricle	78	88	1 10	64	0 05
Transposition of great arteries	82	62	10 41[a]	182	0 21
Tricuspid atresia	91	79	0 00	35	0 04
Truncus arteriosus	95	96	0 31	71	0 09
Ventricular septal defect	31	17	30 19[a]	256	0 30
Tetralogy of Fallot	41	34	0 10	118	0 14
Ebstein s anomaly	0	60	5 10[a]	5	0 01
Other malformations	47	34	1 24	56	0 07
Total	56	35	175 87[a]	2 033	2 38

Table 4 *Frequency of CHD in siblings of 4 333 probands with cardiac malformations*

Main diagnosis	Pro bands	Affected siblings	%
No diagnosis	310	6/420	1 4
Coarctation of the aorta	287	10/398	2 5
Aortic stenosis	203	9/392	2 3
Atrial septal defect	406	16/639	2 5
Persistent ductus arteriosus	639	18/871	2 1
Common atrioventricular canal	41	2/36	5 6
Fibroelastosis + cardio myopathia	104	17/128	13 3
Hypoplastic left heart	134	1/130	0 8
Pulmonary atresia with intact ventricular septum	30	2/45	4 4
Pulmonary stenosis	292	9/501	1 8
Anomalous pulmonary venous drainage	35	0/40	0 0
Single ventricle	65	0/87	0 0
Transposition of great arteries	241	3/272	1 1
Tricuspid atresia	43	1/35	2 9
Truncus arteriosus	64	0/80	0 0
Ventricular septal defect	1 035	23/1237	1 9
Tetralogy of Fallot	263	12/297	4 0
Ebstein s anomaly	16	0/20	0 0
Other malformations	125	6/207	2 9
Total	4 333	135/5835	2 3

(37 6%) when only patients in whom the dia gnosis was established by heart catheterization and/or autopsy are included

The cumulative lethality for all 5 249 children was 25% after 1 month, 42% after 5 years and 48% at the age of 15 years A total of 2 033 children died (a mortality rate of 2 4 per 1 000 children at risk)

Table 3 shows that the lethality during the second part of the period of study (1969–73) was lower (41%) than during the first part Of the 2 033 children who died 1 623 (80%) had an autopsy compared with a figure of 55% for children who have died in general in Denmark in 1968 in the age group 0–15 years The causes of death were cardiac failure, 58 9%, pul monary complications, 16 2% (pneumonia, pulmonary haemorrhage, hyaline membrane disease), extracardiac congenital malforma tions, 12 6%, other causes, 12 3% (including 151 or 7 5% in connection with heart surgery)

Information on CHD in siblings (Table 4) was obtained in 4 333 probands, and 135 cases of CHD were found among 5 835 siblings (2 3%) Affected siblings were born either be fore or after the proband In the case of more probands in one sibship a correction was made for multiple selection CHD was concordant in 60 cases, 26 were not concordant, and in 49 cases the nature of the cardiac anomaly was unknown

DISCUSSION

The prevalence increased during the period of study and this was probably mainly caused by the inclusion of a large number of less severe cardiac defects during the second part of the period Because of the smallness and uniform geography of Denmark no difference in pre valence would be expected in different parts of the country The high prevalence on the island of Funen is probably caused by a better recognition of CHD in the year of question Earlier studies have revealed prevalences be tween 6 and 8‰ (1, 2, 3, 10) A working group of WHO (19) suggested that 8‰ is probably too low, and that the true prevalence is be tween 8 and 10‰, but in obtaining such high values, clinically insignificant malformations, such as bicuspid aortic valves and isolated right aortic arch, are probably included Such anomalies were not included in the present study, as defects which usually do not produce any symptoms in childhood will be found only sporadically in a retrospective study

In the present study 63% of the children were detected within the first year of life, and this figure is rather similar to the results of other authors Carlgren (2) 52%, Feldt et al (5) 86% Rose et al (17) 60%

In 1965 Landtman (11) examined the months of birth of 3 402 cases of CHD in Finland, and he found that more of these were born in late summer and early autumn (8–9 months after the highest seasonal inci dence of acute infectious diseases) as com pared with the population in general In the present study both children with CHD and

normal children were born most often during early spring but no statistical difference could be found And furthermore, as the highest incidence of acute infectious diseases in Denmark (as in Finland) is at the beginning and end of the year it is not possible to show an association between infectious disease in the mother and the occurrence of CHD in the child by the above mentioned method in the present study

The relative frequency of individual cardiovascular malformations seen in Table 2 is rather similar to the results of some other population studies (3 6, 10 15) except in the case of ventricular septal defect The author found 26% during the period 1969–73 and this figure is low in comparison with 42% found by Carlgren (3) No doubt many insignificant cases have escaped recognition in the present retrospective study Hoffman (7) expressed the view that the minimal incidence of ventricular septal defect is 2/1 000 livebirths In the present series it was 1 8 per 1 000 children at risk (period 1969–73)

In comparing the lethality in children with CHD in various studies it is often seen that clinical series are not directly comparable because there may be a difference in time resulting in diverse possibilities for operative treatment The lethality for a total group will also be highly dependent on the number of less severe cases included and especially upon the number of ventricular septal defects Small ventricular septal defects will show a lethality of zero while larger defects may show 40% The lower lethality observed during the second part of the period was partly caused by the inclusion of more less severe cases of CHD but mainly by a better medical and surgical treatment of some malformations (e g transposition of the great arteries) The observed lethality of 35% at the age of 5 years (period 1969–73) is rather similar to the results of Mitchell et al (15) 35%, Hay (6), 41%, Carlgren (3) 34% The earlier mentioned causes of death have to be interpreted with caution because these depend to a great extent upon the judgement of the investigating doctor In many cases, death is a combination of several conditions, especially cardiac failure and pulmonary complications, and it may be difficult to ascertain the most important cause

The risk of CHD in siblings was 2 3% (3–4 times as high as the prevalence in the general population) All children with chromosomal aberrations and children having CHD in association with diseases known to be caused by single mutant genes were excluded from the calculations of risk figures The higher risk in siblings indicates, that genetic factors are involved, and in most cases probably the multifactorial inheritance mechanism (16) The risk of 2 3% corresponds well to the results of other authors basing their results on much smaller series Nora (16), 2 8%, Kenna et al (10) 1 5–2% The high risk figures seen in fibroelastosis are in agreement with the results of Chen et al (4) who found a risk of 17 7% after the birth of the first affected child There may perhaps be different groups of fibroelastosis, some with multifactorial inheritance, some with recessive and some with nongenetic (e g viral) etiology

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Submitted Dec 13, 1979
Accepted March 21, 1980

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Acta Pædiatr Scand 69 625–631 1980

NEUROLOGY AND BEHAVIOUR OF NEWBORN INFANTS DELIVERED BY VACUUM EXTRACTION ON MATERNAL INDICATION

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ABSTRACT Leijon I (Department of Paediatrics, University Hospital, Linkoping, Swe- [illegible]

[illegible] Infants were examined on the 1st and 5th days after birth with the Brazelton Neonatal Behavioural Assessment Scale and by a standardized neurological examination On day 1 the VE-infants showed lower visual and auditory responsiveness in the behavioural assessment, and fewer optimal responses in the neurological examination than did the controls Obstetrical factors such as abnormal presentation, long duration of the second stage of labour, and long duration of VE (≥15 minutes) may explain the differences The differences between the groups had largely disappeared by day 5

KEY WORDS Vacuum extraction, newborn infants, behavioural assessment, neurological examination

Vacuum extraction (VE) has become the predominant method of instrumental delivery in Sweden and elsewhere The incidence of VE deliveries in Sweden increased from 4 2% in 1973 when nation wide statistics first became available, to 6 8% in 1978 (Swedish National Birth Statistics) The mortality rate (3 19) and neonatal morbidity (intracranial haemorrhage retinal haemorrhage abrasions of the scalp etc) associated with VE have been studied (5 6 9 16) Distinction has not always been made between fetal or maternal indications however (2 11 15) so that evaluation of the effect on the newborn infant has been uncertain The neurological condition of neonates born by VE has been reported by Blennow et al (5) but no studies on the behaviour and neurological condition in the early neonatal period have been described

This report compares the behaviour and the neurological condition of a group of neonates delivered by VE on maternal indications with newborn infants born without instrumental

MATERIAL AND METHODS

The VE group was selected over a 3 month period in 1978 at the University Hospital Linkoping In this year 179 (10 2%) of all mothers were delivered by VE 101 (56 4%) VEs being done chiefly on maternal indications For the study a sample of such cases was selected using the following criteria

(i) Medically uneventful pregnancy with the date of the last menstrual period known

(ii) Spontaneous onset of labour at full term (>37 completed weeks)

(iii) Vertex presentation

(iv) Normal fetal heart rate pattern during labour recorded by cardiotochography (CTG) Parturients with late or variable deceleration in heart rate or constant bradycardia or tachycardia were excluded Patients with meconium stained amniotic fluid were excluded

An essential requirement was that VE had been done on exclusively maternal indications (primary or secondary uterine inertia) The VE group consisted of 23 women (18 primiparae 5 multiparae) of whom 2 had primary uterine inertia and 21 secondary uterine inertia

A control group of 16 mothers (8 primiparae 8 multiparae) and their infants seen during the same period was selected Infants who fulfilled the criteria for normality as described above were selected at random 10 of the labours were CTG registered and the others were followed by careful auscultation of the fetal heart sounds There were no abnormal deviations in heart rate and no signs of uterine inertia and the infants were born in

Table 1. *Maternal age, gestational age, duration of labour from cervical dilatation 4 cm until delivery, and duration of second stage of labour*

Means and S D

	VE group	(*n*)	Control group	(*n*)
Maternal age, years	27 7±4 6	(23)	27 6±5 7	(16)
Gestational age, weeks	40 7±1.5	(23)	40 7±1 2	(16)
Duration of labour, min				
primiparae	469±259	(17)	284±212	(7)
multiparae	373±272	(3)	153±92	(7)
Duration of second stage, min		**		
primiparae	61 4±25.5	(15)	28 0±5 7	(8)
		**		
multiparae	59 3±20 5	(3)	21 7±12 2	(7)

** $p<0\ 01$

uncomplicated vertex presentation without instrumental assistance

All women received routine antenatal care The gestational age was confirmed by ultra-sound in early pregnancy The maternal and gestational ages did not differ between the groups The duration of labour fro 4 cm cervical dilatation until delivery and the secon stage of labour were longer in the VE group in bot primiparae and multiparae (see Table 1) The mother delivered by VE received more analgetics and anes thesia, notably paracervical nerve block (70 and 38% respectively), than the controls The difference was no significant, however There were 5 occipito-posterior (OP positions in the VE-group and none in the controls The time from application of the extractor cup to birt was 16 7±6 6 min, range 9–28 min (in 8 cases ≥15 min) The mean number of extractions was 3 2 (range 1–10) 9 infants were extracted by mid-VE, the cervix fully dilated and the head at the level of the spines, 14 wer extracted by low VE, the cervix fully dilated and the hea below the spines In 8 cases the head was not fully rotate when the extractor cup was applied

Table 2. *Items in the behavioural assessment and the subscales*

Subscale	Item
Habituation items (*n*=4)	Response decrement to repeated light stimuli repeated rattle stimuli repeated bell stimuli repeated pinpricks
Orientation items and alertness (*n*=6)	Orientation to object Orientation to sound Orientation to face Orientation to voice Orientation to face and voice Alertness
Motor items (*n*=5)	General tonus[a] Motor maturity Pull-to sit Defensive movements Activity level[a]
Range of state (*n*=4)	Peak of excitement[a] Rapidity of build up[a] Irritability[a] Lability of state[a]
Regulation of state (*n*=4)	Cuddliness Consolability Self quieting activity Hand-to mouth activity
Physiological stability (*n*=2)	Tremulousness[a] Startles[a]

[a] 'Unlinear' item rescored (Lester et al , personal communication)

weight gain started All infants remained with thei mothers on the maternity ward throughout the neonata period

The infants were examined at the ages of 7–28 hours mean 19 hours (day 1) and on day 5 with the Brazelton Neonatal Behavioural Assessment scale (7) For statis tical analysis some items were rescored (Lester et al , personal communication) so that high scores always indi cated optimali [illegible] (Lester et al [illegible] was calculatec [illegible] see Table 2) The initial and predominant states were observed during the examination as directed (7) Some items could not always be assessed The mean for a subscale could not be calculated if one item was missed

A neurological examination mainly as described by Prechtl and Beintema (17) was done at the same time This included 36 items most estimated on a four point scale (14) A muscle tonus score and an excitability score (18) were calculated from items reflecting mainly these

Table 3 *Behavioural subscales and initial and predominant states*

Mean ($\bar{x}$) and standard deviation (S D) in both groups on day 1 and 5 Unlinear items were rescored Higher values for subscales indicated a more optimal performance

	Day 1						Day 5					
	VE infants			Controls			VE infants			Controls		
	$\bar{x}$	S D	(n)	$\bar{x}$	S D	(n)	$\bar{x}$	S D	(n)	$\bar{x}$	S D	(n)
Habituation items	7 7±0 7		(18)	7 4±0 7		(7)	7 3±0 6		(20)	6 8±1 1		(8)
Orientation items + alertness	5 1±1 2		(22)	6 0±0 7		(15)	5 5±1 1		(23)	6 1±0 6		(15)
Motor items	4 7±0 8		(23)	5 0±0 5		(15)	5 7±0 6		(23)	5 7±0 5		(14)
Range of state	4 1±0 7		(23)	3 9±0 7		(14)	3 9±0 8		(23)	3 9±0 5		(15)
Regulation of state	4 9±1 1		(19)	4 7±0 8		(12)	4 6±1 1		(21)	4 5±1 2		(13)
Physiological stability	7 2±1 7		(20)	6 8±1 1		(15)	8 1±0 6		(23)	8 1±0 8		(15)
Initial state (range 1–6)	2 0±0 9		(23)	1 5±0 5		(16)	1 7±0 6		(23)	1 9±0 5		(16)
Predominant state (range 1–6)	4 7±1 3		(23)	4 6±1 2		(16)	5 0±1 1		(23)	5 4±0 5		(16)

Significance brackets: Orientation items, VE day 1 vs Controls day 1 *; Orientation items, VE day 1 vs VE day 5 ***; Orientation items, Controls day 1 vs Controls day 5 *; Physiological stability, VE day 1 vs VE day 5 *; Physiological stability, Controls day 1 vs Controls day 5 ***; Initial state, VE day 1 vs Controls day 1 *

*$p<0.05$ *** $p<0.001$

factors High values indicated low muscle tonus and low excitability respectively The number of optimal responses (i e items for which an optimal response can be obtained) and a total neurological score were also calculated for each infant High values for total neurological score mainly indicated low tonus and/or low excitability

Conventional statistical methods were employed Student s t test was used throughout in comparisons between the groups Levels of significance refer to two-tailed tests When comparing groups account was taken of differences concerning parity Multiple regression technique was used in some analyses

The investigation was approved by the Ethical Committee of the Faculty of Medicine University of Linkoping Informed consent was given by all mothers

RESULTS

When comparing groups allowance was made for parity, which did not explain the differences presented below Birthweight, length, head circumference and postnatal weight loss did not differ between the groups In the VE group 2 infants (8 7%) had minor cephalhaematoma and 3 (13 0%) scalp abrasions caused by the extractor cup The mean Apgar score at 1 min was 8 5±1 1 for the VE-group and 9 1±0 3 for the controls ($p<0.05$) One infant in the VE group showed mild postnatal asphyxia (Apgar score 4 and 8 at 1 and 5 min), the other infants had one-min Apgar scores of 7 or more There were no differences in mean Apgar score at 5 min In 8 (34 8%) of the VE infants and 2 (12 5%) of the controls the serum bilirubin concentrations during the neonatal period exceeded 180 µmol/l The difference in incidence between the groups was not significant

The individual items in the behavioural examination showed in the VE-group significantly lower scores for the 5 orientation items on day 1 and for 3 orientation items on day 5, compared to the controls ($p<0.05$) With regard to other items there were no significant differences The initial state was higher in the VE-group on day 1 ($p<0.05$) than in the controls, but the predominant state did not differ There were more missing data among habituation items in the controls than in the VE-group, missing data in other items were few in both groups Table 3 gives the results for the

Table 4. *Results of the neurological examination*

Mean ($\bar{x}$) and standard deviation (S.D.) in both groups on day 1 and 5. High values in tonus score or in excitability score mean low muscle tonus or low excitability respectively

	Day 1				Day 5			
	VE infants (n=23)		Controls (n=16)		VE infants (n=23)		Controls (n=16)	
	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.
Tonus score (optimal=14.0)	13.4±1.6		13.8±0.8		14.1±0.6		14.0±0	
Excitability score (optimal=14.0)	15.2±1.8		14.3±1.0		14.2±0.5		13.9±0.3	
Number of optimal items (maximum 27)	24.2±2.6		26.3±1.1		26.3±1.2		26.9±0.3	
Total neurological score	53.3±3.0		51.1±1.5		51.7±1.3		50.9±0.3	

*$p<0.05$ **$p<0.01$ ***$p<0.001$

6 behavioural subscales. The VE-infants had a significantly lower mean score for the orientation subscale (i.e. lower visual and auditory responsiveness) on day 1 ($p<0.05$), but the difference was not significant on day 5. The remaining subscales showed no differences. There was significantly better performance in motor items and better physiological stability, i.e. less tremulousness and startles, in both groups on day 5 than on day 1.

In the neurological examination, the VE-infants showed significantly fewer optimal items on day 1 and a higher total neurological score on day 1 and day 5 than the controls (Table 4). There were no significant differences in mean tonus score or mean excitability score between the groups. The incidence of low excitability (arbitrarily defined as a score of 16 or more) was greater in the VE group ($p<0.05$). On day 5 there were few significant neurological differences between the groups. The VE infants showed significantly better results with regard to the four neurological categories on day 5 than on day 1.

The infants presenting abnormally (5 OP positions in the VE group) and infants born after VE lasting 15 min or more (8 infants, 2 of them born in OP position) differed significantly in some behavioural and neurological variables from the controls and the remaining VE-infants (Table 5). The most striking findings in the *behavioural* assessment were lower mean for the orientation subscale on day 1 for infants presenting with OP and for infants with prolonged VE (≥15 min) compared with the controls. The remaining VE infants (occipito-anterior (OA) positions with VE-duration <15 min) did not differ significantly in this subscale from the controls. On day 1 infants born in OP positions showed in the *neurological* examination lower excitability and fewer optimal items than those born in OA positions and the controls. Infants born after prolonged VE had fewer optimal items on both days than the controls. Infants with OA positions and short VE-duration (<15 min) did not differ significantly in neurology from the controls.

Multiple regression analysis was done to analyse further the effects of the time variables. The duration of labour (from 4 cm cervical dilatation until delivery) was not significantly related to the behaviour or neurological condition on both days. The duration of the second stage of labour was

Table 5 *Mean of the orientation subscale and the neurological summary scores in 3 groups of VE infants (occipito posterior (OP) position, occipito-anterior (OA) position with VE-time ≥15 minutes, and OA position with VE-time less than 15 minutes) and controls*

	OP position (n=5)	OA position VE time ≥15 min (n=6)	OA position VE time <15 min (n=12)	Controls (n=16)	Standard deviations within groups
Orientation items + alertness Day 1	4 5	4 7	5 6	6 0	0 98
Day 5	5 7	4 9	5 7	6 1	0 89
Tonus score (optimal 14 0) Day 1	13 0	13 3	13 7	13 8	1 32
Day 5	14 0	14 0	14 3	14 0	0 46
Excitability score (optimal 14 0) Day 1	16 8	14 8	14 8	14 3	1 45
Day 5	14 4	14 2	14 2	13 9	0 45
Number of optimal items (maximum 27) Day 1	22 0	24 2	25 1	26 3	1 91
Day 5	26 6	25 5	26 6	26 9	0 94
Total neurological score Day 1	56 0	53 2	52 3	51 1	2 29
Day 5	51 4	52 2	51 7	50 9	1 05

*p<0 05 **p<0 01 ***p<0 001

significantly related to the number of optimal neurological items, excitability scores (low excitability), and total neurological scores on day 1 The duration of VE showed additive effects with regard to the number of optimal neurological items on day 1 The duration of VE was also significantly related to the mean for the orientation subscale on day 1 The duration of second stage or the duration of VE were not significantly related to behaviour or neurological findings on day 5 Multiple regression analysis also showed no significant relationships between the head circumference, the station of the head at the time of application of the cup, or the mode of analgesia on the one hand and the behavioural or neurological variables on day 1 on the other. Peak serum bilirubin concentrations exceeding 180 μmol/l were not significantly related to the behavioural or neurological outcome on day 5 on multiple regression analysis

DISCUSSION

VE has been extensively used in obstetrics for about two decades, but little is known of

its possible effects on the cerebral function of the newborn infant It is not possible to obtain two randomized groups in a study of this type, because VE cannot be used in normal deliveries, for ethical reasons Nevertheless, maternal factors such as age, health, normal course of pregnancy, and gestational age were identical in the two groups In the present study the indications for VE were only primary or secondary uterine inertia, with no signs of fetal asphyxia before the extraction As in most other studies on VE (for review see (20)) primiparae outnumbered multiparae In the statistical analyses allowance was made for this divergence Differences with regard to anaesthesia probably do not explain any of the differences found, since multiple regression analysis disclosed no influence of analgesia or anaesthesia on the behaviour or neurological condition of the infants

The observed differences between the VE-infants and controls concerning Apgar scores at 1 min and the neurological and behavioural examinations on day 1 can be explained by mild prenatal asphyxia According to Hickl (10) the mechanical pressure on the fetal head by the extractor may cause cerebral hypoxia and depression of the central nervous system Ibanez et al (12) explained the high frequency of retinal haemorrhage also reported by others (2, 9, 11 13) to be caused by spasm of the lower uterine segments, leading to considerable pressure on the fetal head and impaired cerebral circulation and hypoxia Blennow et al (5) showed an increased number of red blood cells in cerebrospinal fluid of VE infants, but in the same study neurological examination on day 4 showed no difference between VE-infants and infants born by spontaneous delivery, irrespective of the indication for VE The neurological examination in Blennow's study is comparable to the optimal neurological items in the present study but because the infants were not examined on day 1, early deviations may have been overlooked Amiel-Tison (1) concluded that labour dysfunction such as OP positions may adversely affect the fetus and may be linked with a hig[h] incidence of abnormal cerebral signs in th[e] neonatal period In the present VE serie[s] there was a high incidence of OP positions these infants showed more behavioural an[d] neurological anomalies on day 1 than did th[e] controls or VE-infants born in OA positions

The duration of labour measured as th[e] time from 4 cm cervical dilatation to birth wa[s] longer in the VE group but was not related t[o] the behavioural and neurological findings Th[e] duration of the second stage of labour and th[e] duration of VE, on the other hand, wer[e] important factors According to Plauch[e] (16), the VE-time should not exceed 45 min The optimal time for the fetus is probabl[y] much shorter an increased incidence o[f] retinal haemorrhage was reported when the extraction time exceeded 20 min (10) In the present study infants extracted in less than 1[5] min from application of the cup did not diffe[r] in any respect, from the controls with regar[d] to behaviour or neurological condition at 1 and 5 days of age The VE infants born afte[r] an extraction time of more than 15 min con[tributed] tributed to lower orientation capacity and t[o] lower number of optimal neurological items the day after birth The differences found be[-] tween VE-infants and controls are thus appa[-] rently attributable to abnormal presentations and/or prolonged second stage including prolonged extraction and not to the use of VE itself

Although statistically significant, the differences between the infants born by VE and the spontaneously born infants were minor and probably without consequence for the future neurological development of the infant Several studies (4 5 8 21) have shown that at follow up examination vacuum extracted children do not differ from spontaneously born children with regard to neurological condition or subsequent development

It may be concluded that using extensive tests of neonatal behaviour and neurological condition newborn infants born by VE on maternal indication without fetal asphyxia

were more often neurologically non optimal and showed lower visual and auditory responsiveness than spontaneously born infants Most differences were transient and could usually be explained by factors such as abnormal presentation of the head, prolonged second stage of labour or prolonged VE

ACKNOWLEDGEMENT

This work was supported by grants from Forenade Liv Mutual Group Live Insurance Company Sweden and by the Medical Faculty Linkoping University Sweden

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Submitted Jan 11 1980
Accepted March 10 1980

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Submitted Jan 11 1980
Accepted March 10 1980

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Acta Pædiatr Scand 69

β_2-MICROGLOBULIN IN CEREBROSPINAL FLUID FROM CHILDREN WITH DIFFERENT DISEASES

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years old 1 ... meningitis values of ...
μg/l (x ≈ 10 ...
in a 13 day ...
values four ...
exceeding ...
sepsis Some correlation between the concentrations of β_2 microglobulin ... found in the diagnostic groups as a whole, while this correlation disappeared when considering each patient individually The significance of β_2-microglobulin as a guide in serious infections is discussed

KEY WORDS β_2-microglobulin, cerebrospinal fluid, virus meningitis, children

β_2 microglobulin (β_2 m) was first isolated from the urine of patients with renal tubular malfunction by Berggård & Bearn (1) The protein has a molecular weight of 11 800 and consists of a single polypeptide chain High serum concentrations were observed in patients with decreased glomerular filtration rate and in some patients with malignant disease The function of β_2 m is still unknown

β_2 m is synthesized and secreted by many types of normal and malignant cells of hematopoietic mesenchymal or epithelial origin There seems to be no correlation between the ability to synthesize immunglobulin and β_2 m

High concentrations of β_2 m have been found in cerebrospinal fluid (CSF) from children with bacterial meningitis (0 44±0 17 mg/100 ml) and medium concentrations in children with viral meningitis (0 20±0 06 mg/100 ml) or low values in healthy children (0 11±0 05 mg/100 ml) (3)

The purpose of this study was to investigate the diagnostic value of β_2 m in CSF in a prospective study

MATERIAL AND METHODS

The study group was comprised of 56 children (16 girls

...toms suggesting meningitis The fluid was kept frozen at −20° until analysis The quantitative determination of β_2 m was performed by means of the radioimmunoassay Phadebas® β_2 microtest (2)

Only patients with normal kidney function (normal values of serum creatinine) were included in the investigation β_2 m was not determined in serum since children with infectious diseases have been found previously to have normal serum values of β_2 m (5)

Table 1 *The age and sex distribution of the patients*

Age	Male	Female
0–6 months	12	6
7–12 months	11	4
13–24 months	4	2
2–4 years	5	2
5–10 years	5	2
11–12 years	3	0
Total	40	16

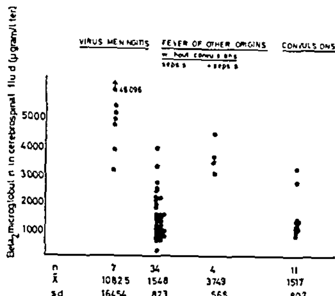


Fig 1 Concentration of β_2 microglobulin in cerebrospinal fluid in different diseases

RESULTS

Viral meningitis was diagnosed in 7 patients because of rise in the antibody titre in blood, positive virus culture, or high mononuclear cell counts in the spinal fluid All the patients with viral meningitis had concentrations of β_2-m of at least 3 000 μg/l in their spinal fluid (Fig 1) The highest value (48 096 μg/l) was found in a 13-day-old infant patient who was infected with herpes simplex virus

None of the patients with fever of other origin had values exceeding 3 500 μg/l, except for one patient with facial nerve paresis and 3 patients with sepsis

The results for β_2-m were compared with the albumin concentrations in the cerebrospinal fluid

The concentration of albumin in CSF was high in the viral meningitis group ($\bar{x}$=31 5 μmol/l, normal values 1 5–5 5 μmol/l) and in the group of patients with sepsis ($\bar{x}$=7 2 μmol/l), while low values were found in the other patients ($\bar{x}$=2 1 μmol/l)

Thus, there appeared to be some correlation between the concentrations of β_2-m and albumin for the different diagnostic group

There was no correlation, however, when results for individual patients in the viral meningitis group were examined The patient with the highest concentration of β_2-m (48 096 μg/l) had an albumin concentration of 18 μmol/l, while the mean for the group was 31 5 μmol/l, and the maximum albumin concentration in this group was 94 5 μmol/l in a patient with a β_2-m value of 5 226 μg/l

DISCUSSION

Although the biological function of β_2 m protein is unknown, interest was focused on this component once its amino acid sequence was shown to have considerable degree of homology with the constant region of various human immunoglobulin polypeptide chains (6) Nevertheless, β_2-m is immunologically unrelated to the immunoglobulins Normal serum values of β_2-m have been found in patients with common infectious diseases, while high serum values have been found in patients with infectious mononucleosis (4, 5)

High values of β_2-m in CSF have been found in patients with bacterial meningitis (3) and in patients with virus meningitis and sepsis In the present study the most striking elevation was found in a child with herpes simplex meningitis who had a β_2-m value 50 times greater than the normal value

On the other hand, low values of β_2-m in CSF were found in children with fever of other origin

It is apparent that β_2-m synthesis and its increased concentration in CSF may reflect serious pathological changes, but more patients must be studied before any conclusion as to the diagnostic value of this protein can be drawn Nevertheless, it seems likely that it may be a guide in diagnosis

ACKNOWLEDGEMENT

I am grateful to Dr K W Kastrup for help in starting this project to Miss A Jacobsen for valuable secretarial help and to Mrs K Jørgensen and Mrs A Wilson for technical assistance

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Acta Pædiatr Scand 69 637–641, 1980

PLASMA FERRITIN CONCENTRATIONS IN PRETERM INFANTS IN CORD BLOOD AND DURING THE EARLY ANAEMIA OF PREMATURITY

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ABSTRACT Hågå, P (Department of Paediatrics and Paediatric Research Institute, National Hospital of Norway, and Department of Paediatrics, Oslo City Hospital, Ulleväl, Oslo, Norway)) Plasma ferritin concentrations in preterm infants in cord blood and during the early anaemia of prematurity Acta Paediatr Scand, 69 637, 1980.—Ferritin concentrations in cord blood were determined in 22 normal term and 32 preterm infants (birth weights 600–2000 g) Eight of the preterms were SGA infants AGA preterm infants had significantly lower concentrations than term infants, and the SGA preterm newborn had even lower levels Plasma ferritin in cord blood of the term and AGA preterm infants [illegible] with plasma iron and transferrin saturations, but not with the trans- [illegible]

ferritin rose rapidly during the first days after birth, peak levels being reached at [illegible] weeks Thereafter linear falls (semilog) occurred with similar slopes in different infants Transferrin concentrations showed a slow progressive increase from 0–8 weeks Plasma ferritin, after reaching the peak value, correlated negatively with weight gain No infant had low ferritin values indicating iron deficiency during the early anaemia

KEY WORDS Cord blood, premature infants, plasma ferritin, early anaemia of prematurity

The concentration of Ferritin in plasma, or serum, has been shown to reflect the iron stores of the body in most conditions, iron deficiency is the only condition that shows abnormally low serum ferritin values (16)

In term infants the cord serum ferritin levels have been found to be high compared to the rest of childhood The serum concentration increases further till 4–6 weeks of age whereafter a fall occurs throughout the first year (10 11 14) Thus also in the newborn period and throughout infancy the serum ferritin seems to reflect the stores although its validity in cord blood is not definitely established (1)

Preterm infants have diminished iron reserves at birth (7), and their very high rate of growth requires large amounts of iron for the expansion of the red cell mass Seip & Halvorsen (13) found that infants with a birth weight less than 1 400 g deposited less stainable iron in their bone marrows in the first weeks of life, and became haemosiderin-free earlier (6–8 weeks) than heavier premature infants Term infants on the other hand had depleted their stores at the age of 20–24 weeks

The premature infant has at birth and in the newborn period, a limited capacity of protein synthesis This may influence plasma concentrations as much as the regulatory factors that are responsible for the plasma levels later in life The purpose of this study was to determine if plasma ferritin in preterm infants at birth and in the newborn period conforms to the known variations in iron stores and thus reflects these stores, secondly, to follow the plasma ferritin concentrations throughout the early anaemia of prematurity in very low-

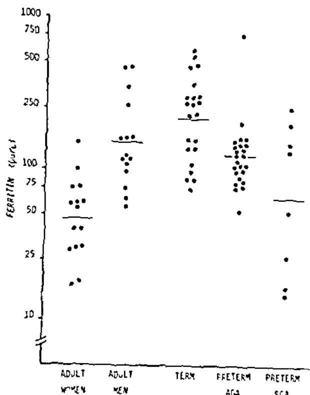


Fig 1 Plasma ferritin concentrations in cord blood of normal term and preterm AGA and SGA infants with birth weights less than 2 000 g The concentrations in normal adult men and women are also shown The horizontal lines are the means of the groups

birth-weight infants supplemented with iron, and thus to assess the effectiveness of the supplementation The infants were given 30 mg Fe^{++}/day from 4 weeks of age, which is the recommended dosage in Norway This regimen is very similar to that used by Gairdner et al (5) in the only study showing any effect of iron medication on the early anaemia of prematurity In their study 36 mg Fe^{++}/day was given from about the third week of life

MATERIALS AND METHODS

Cord blood samples were obtained from 22 normal term and 32 preterm infants The preterm infants had birth weights ranging from 600–2 000 g and were scored as appropriate for gestational age (AGA) (n=24) or small for gestational age (SGA) (n=8) according to the charts prepared by Gairdner et al (6) Sixteen of the samples were heparinized (Vacutainer® Becton Dickinson) while the rest were collected as whole blood Heparin does not

parison with the newborn values

Seventeen AGA premature infants were followed at weekly intervals from admittance to the neonatal intensive care unit until discharge Their birth weights ranged from 850–1 500 g and they all had uncomplicated neonatal periods except for short apnoeic spells The infants were fed a commercial cow s milk formula (Nan Nestle) that contains 5 5 mg iron/l whereas the linoleic acid content is 12 % of the total amount of fat After two weeks or later nine of them started to receive milk from their own mothers From the fourth day of feeding the infants were supplemented with vitamin A C and D folic acid and vitamin E (7 5 mg water soluble α tocopherol/day) Iron (ferro carbonate equivalent to 30 mg Fe^{++}/day) was given from the fourth week of life Blood was collected by venipuncture at the age of one and four weeks and then every second week Samples taken at other times were obtained by heel puncture The samples were collected into heparinized plastic tubes Blood was always sampled in conjunction with routine haematologic assessment of the preterm infants Ferritin was measured by RIA Gnost® Ferritin (Behring Institut) In order to reduce the required volume of plasma needed for the measurements the method was modified as follows The beads containing antiferritin were incubated for 24 hrs/37°C with 25 µl of standards or test sera/plasma and 125 µl of the buffer The beads were washed and then incubated for a second 24-hr period (37°C) with 150 µl of ^{125}I antiferritin After washing the radioactivity of the beads was counted (3×4 min) Test samples and standards were done in triplicate The intra assay coefficient of variation ranged from 1 2 4 0 % when five samples that covered the range of the standard curve were tested six times An inter assay coefficient of variation of 11 9 % was obtained when a normal adult plasma was measured in 16 consecutive assays According to the manufacturer a ferritin concentration of 14 µg/l or lower measured with this kit constitutes latent iron deficiency in adults

Plasma iron was measured on a Technicon Auto Analyzer the method being scaled down to 180 µl per sample Transferrin concentrations were determined by single radial immunodiffusion (M Partigen Behring Institut) Plasma vitamin E concentrations were also measured and will be reported elsewhere Haemoglobin haematocrit red blood cell and reticulocyte counts were determined by standard laboratory methods The transferrin saturations were calculated by converting the transferrin measurements to TIBC by using the conversion factor obtained by Tsung et al (15)

Student s t test was used for the statistical evaluation the ferritin values transformed to logarithms as ferritin in normal subjects show a log normal distribution (14)

RESULTS

Cord blood

Fig 1 shows the ferritin concentrations in cord blood of term and preterm newborn as well as those of normal adult men and women The term infants have as reported by others (10,

Table 1. Cord plasma concentrations of ferritin, iron and transferrin, and transferrin saturations in normal term and preterm SGA and AGA infants with birth weights 600–2 000 g

Results are means ± S.D. The number of infants investigated is in parenthesis

	Ferritin (μg/l)	Plasma iron (μmol/l)	Transferrin (g/l)	Transferrin saturation (%)
Preterm AGA	125[b] (76–205) ($n=24$)	16.8±9.0[c] ($n=24$)	1.60±0.39[c] ($n=23$)	48±20 ($n=23$)
Preterm SGA	67[b] (21–210) ($n=8$)	18.3±9.6[a] ($n=7$)	2.39±0.61 ($n=7$)	41±22 ($n=6$)
Term	218 (115–413) ($n=22$)	27.1±6.7 ($n=21$)	2.24±0.42 ($n=22$)	55±14 ($n=21$)

(4) values of the same magnitude as adult men. The levels in preterm AGA infants are significantly lower ($p<0.005$) than those of term infants. Preterm SGA infants have even lower levels and the variation seems to be larger, with some having quite low concentrations. In this group of AGA preterms with birth weight less than 2 000 g the ferritin concentrations did not correlate significantly with gestational age nor with birth weight. When term infants are included the correlation with birth weight becomes significant ($r=0.50$, $p<0.001$).

Table 1 shows the concentrations of ferritin, plasma iron, transferrin, and transferrin saturation in cord blood of AGA and SGA preterm and normal term infants. In addition to the lower ferritin values the levels of plasma iron are significantly lower in the preterm infants. Transferrin concentrations are significantly lower in AGA preterm than in term infants, while the SGA preterms have similar transferrin levels as term infants. There is no difference in transferrin saturations between the groups.

The ferritin concentrations in cord blood of term and preterm AGA infants correlated positively with serum iron concentrations ($r=0.49$, $p<0.001$) as well as with transferrin saturations ($r=0.38$, $p<0.02$), while there was non-significant correlation with transferrin. On the other hand serum iron and transferrin showed a highly significant positive correlation ($r=0.60$, $p<0.001$).

Early anaemia of prematurity

The changes of plasma ferritin during the early anaemia of prematurity for AGA preterm infants with birth weight less than 1 500 g are shown as individual curves in Fig. 2. After birth, the ferritin concentrations increase rapidly, the peak levels occurring at 1–4 weeks of age in different infants. Thereafter, the concentrations fall linearly (semilog), the slopes of the individual curves being surprisingly similar. Iron medication started 4 weeks after birth seems not to affect the course of the curves. No infant had low ferritin concentrations indicating depleted iron stores during the early anaemia. The ferritin values (log), as the percentage of the individual's maximal value, and weight gain, as the percentage of the weight at the time of the maximal ferritin value, displayed a highly significant negative correlation ($r=0.81$, $p<0.001$).

The haemoglobin concentrations of the preterm infants showed the well known fall, with nadir reached at 6–8 weeks of age, at the same time the reticulocyte counts were maximal.

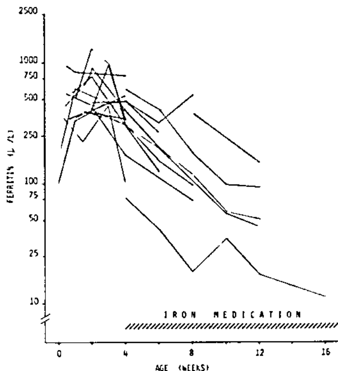


Fig 2 Ferritin concentrations in plasma of 16 AGA preterm infants (birth weights 850–1 500 g) versus age Each line represents one child Iron medication (30 mg Fe^{++}/day) was given from 4 weeks of age

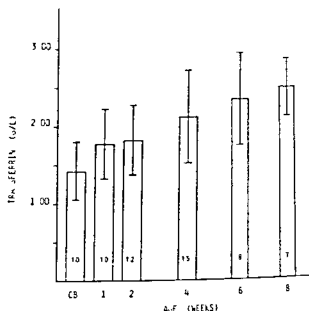


Fig 3 Transferrin concentrations (means±S D) in plasma of AGA preterm infants (birth weights 850–1 500 g) versus age The number within the bars refer to the number of infants in each group

The plasma iron levels as well as the transferrin saturations did not change significantly during the same period On the other hand, transferrin concentrations showed a gradual increase from 0–8 weeks as shown in Fig 3

DISCUSSION

In this study cord blood ferritin concentrations were significantly lower in preterm than in term infants As in the adult organism this probably indicate lower iron storage concentrations in preterm than in term infants Haemoglobin iron is, however, the most important iron pool of the newborn for subsequent growth, since approximately 75% of the iron is in the red cell mass (7)

The iron in storage depends on the amount transported across the placenta, and the rates of growth and erythropoiesis of the foetus The transfer of iron increases with increasing gestational age (4), while the foetal growth rate (6, 8) and rate of erythropoiesis (17) fall during the last weeks of pregnancy. The preterm infant at birth thus incorporates relatively more of its iron into haemoglobin than at term The lower ferritin concentration reflects this situation The even lower ferritin concentrations in SGA infants who have a higher erythropoietic activity (3), fit well with this concept

preterm, but this is unlikely since the ferritin levels rise rapidly after birth This is in contrast to the gradual rise in transferrin concentrations during gestation (12) and the slow rise after birth (Fig 3), indicating that protein synthesis capacity is an important determinant for the transferrin, but not for the ferritin concentration

The significant positive correlation between cord plasma ferritin and plasma iron of AGA preterm and term infants is in agreement with data obtained for adults by Bezwoda et al (2)

The rapid rise in ferritin concentrations seen after birth reflects the shut off of erythropoiesis after birth The levels continue to rise during the neonatal period because the iron liberated from erythrocyte breakdown is stored and red cell production is still negligible The mean level of plasma ferritin was maximal at 2 weeks of age, at which time the reticulo-

cyte counts were still low The fall in ferritin concentrations occurring from that time on coincides with the resumption of erythropoiesis as judged from the increasing reticulocyte levels These date are in good agreement with the early data of Seip & Halvorsen on iron stores during this period (13), as well as the findings of Lundstrom et al (9) on the course of serum ferritin in low birth weight infants The latter study evaluated iron supplementation of 2 mg/kg/day from the second week of life in infants with birth weight less than 2000 g Low ferritin levels indicating depleted iron stores were found in about 15% of the supplemented infants at 3 months of age The depletion though was transitory in most of the infants

Linear (semilog) declines as seen in plasma ferritin during the early anaemia are often found when one factor is responsible for the changes observed The regulation of erythropoiesis at this age is very complicated However the highly significant negative correlation found between ferritin concentration and weight gain points to the primary importance of growth

The Norwegian regimen of starting supplementation at 4 weeks of age is based on the early findings of depleted stores at 6–8 weeks of age in the very low birth weight group evaluated by bone marrow staining (13) Judged from the plasma ferritin values obtained in this study iron deficiency seems not to occur during the first 12 weeks of life on this regimen

ACKNOWLEDGEMENTS

The scaled down automated method for plasma iron measurement as well as the actual determinations was the excellent work of Åge Stensberg Some of the cord blood samples of the preterm infants were collected by Alf Meberg M D and I an grateful for being allowed to analyze them This study was supported by a grant from The Norwegian Research Council for Science and the Humanities

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Submitted Oct 5 1979
Accepted Febr 6 1979

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Acta Pædiatr Scand 69 643–646, 1980

INCREASED EXCRETION OF A BRAIN DEPRESSOR AMINE IN INFANTILE COELIAC DISEASE AND IN HEALTHY INFANTS ON A HIGH PROTEIN MILK DIET

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ABSTRACT Lindblad, B S and Rafter, J J (Department of Paediatrics at St Goran s Children s Hospital and the Department of Medical Nutrition, Karolinska Institutet, Stockholm, Sweden) Increased excretion of a brain depressor amine in infantile coeliac disease and in healthy infants on a high protein milk diet Acta Paediatr Scand, 69 643, 1980.—Urinary excretion of piperidine, a heterocyclic pressor amine of gut bacterial origin and nicotine-like activity in the brain, has been estimated by a gas chromatography method in healthy men and women, in normal breast fed and formula fed infants and in infants with untreated coeliac disease The excretion of piperidine cannot usually be detected during the first [illegible] k of life The amount present in urine increases upon weaning with higher excre- [illegible] responsible for the initial mental depression commonly seen in this disease and suggests that piperidine is one of the ' auto-intoxicating ' substances arising from the bacterial decomposition of protein postulated by Metchnikoff in 1903 but hitherto unidentified

KEY WORDS Piperidine, biological amines, mental depression, transmitter substances, protein nutrition, coeliac disease, brain metabolism, intestinal flora

Mental depression is commonly observed in infants and small children during the initial phase of untreated coeliac disease The mothers often comment upon the rapid change of mood in their child after the initiation of treatment with a gluten free diet It seems to be worth finding out if these infants, during the phase of clinical malabsorption, show increased absorption of toxic substances from the non absorbed components of their diet (18) This could explain the change of mood observed during the course of treatment

Piperidine was chosen for study from the various biologically active substances which are known to be of bacterial origin and found in normal urine (17) The reasons for the choice were (*a*) it is the dominant pressor substance of normal human urine (7) and a normal constituent of brain and the cerebrospinal fluid (14), (*b*) while the endogenous formation of piperidine is still a matter of speculation, the bacterial decarboxylation of dietary lysine and the subsequent production of piperidine (Fig 1) is well established (3), fecal bacteria convert (^{14}C) lysine *in vitro* to radioactive piperidine (2), (*c*) antibiotic treatment considerably decreases its fecal and urinary concentrations (3, 20), suggesting that the major part is derived as a result of bacterial action, and (*d*) its recognized effects on brain function (10) The increased excretion of piperidine in cystinuria (19), an inborn error of metabolism with malabsorption of lysine, also indicates the possible utility of this biochemical system in our investigation

METHODS

Urine was collected after washing of the genitalia as for urinary bacterial culture and was frozen less than 10 min after micturition The sample was kept at −20°C until

This paper is written in honour of Professor Rolf Zetterstrom on his 60th birthday

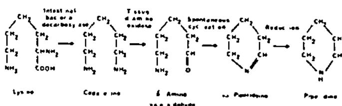


Fig 1 Metabolism of lysine to the diamine cadaverine (2, 8) and to the heterocyclic secondary amine piperidine (2 3)

it was analyzed for heterocyclic amines by gas chromatography of their dinitro phenyl derivatives (17) The derivatization and the cyclohexane extraction was performed according to Asatoor & Kerr (4) The variation coefficient of the method was ±8°7

Piperidine and pyrrolidine were identified by gas chromatography—mass spectrometric analysis of some samples (17) Creatinine was determined colorimetrically by the Jaffe reaction (6) The TCA deproteinized sample was treated with Fuller earth (Lloyd s reagent) in order to eliminate as far as possible non specific cromogens The mean of two determinations was used and the variation coefficient was as low as 0 4% The statistical analysis was performed by the paired *t* test

MATERIAL

Twelve men and 12 women working at the paediatric department who were subjectively and apparently healthy and who had not received antibiotics for the last 6 months were included in the study as adult controls

Six breast fed and 4 formula fed premature infants were studied during their uncomplicated stay in the premature nursery The age distribution was the same in the two groups, ranging from 2–6 weeks Seventeen infants were studied at the maternity ward Their pregnancy and delivery was normal and they all received their mother s milk with substitution of breast milk from a milk bank in smaller amounts during the first three days when needed The same infants were studied at 1 2 months of age (8 cases) and at 4–6 months of age (8 cases 4 still completely breast fed and 4 formula fed) Eleven infants were studied at 1 year of age, they were then all consuming a normal diet for age out of the families ordinary diet All these normal infants showed a normal speed of growth from birth and had not been subjected to antibiotic treatment

Fourteen infants, 6 months–1 year of age, were studied during an investigation and follow up for suspected malabsorption syndrome due to suboptimal growth Out of these 14 patients 5 were subsequently found to suffer from coeliac disease and 9 were freed from suspicion of mal absorption altogether through negative jejunal biopsy and a normal follow up In one coeliac case (A D, G 31) with severe growth retardation, constipation and mental depression one sample was taken at hospitalization and one upon partial rehabilitation to normal speed of growth and normal mental state on a gluten free diet

RESULTS

Results are summarized in Table 1 Adult women show a higher mean than men, but

Table 1 *Urinary excretion of piperidine (mmol/m creatinine) in healthy infants, adults and untreated coeliac disease of infants*

Age	*n*	Mean	S E M	*P*	Undetected in *n*
Premature infants					
2–6 weeks					
Breast fed	6	–	–		6
Formula fed	4	1 98	1 0		
Term infants					
0–6 days	17	0 29	0 09	0 001	10
1–2 months	9	1 93	0 30		1
4–6 months					
Breast fed	4	1 65	0 55	0 01	
Formula fed	4	5 95	0 56	0 005 (Formula fed vs 1–2 years)	
1–2 years	11	1 63	0 38		
Adult men	12	0 56	0 14		
Adult women	12	1 54	0 49		
Infants					
6 months–1 year					
No malabsorption	9	1 11	0 95	0 01	
Coeliac disease	5	3 62	1 40		

since they show considerably greater variation the mean difference is not significant

There was a highly significant difference between the almost non existent urinary piperidine content between 0–6 days of life and at one to two months of age, when it equals that of normal adults At 4–6 months a significantly higher piperidine content was found in formula fed than in breast-fed infants In premature infants fed on human milk, the urinary content rises from undetectable amounts to normal for age on weaning The subsequent concentrations of piperidine in the urine returns to normal adult levels at 1–2 years of age

There was a significant difference between the urinary piperidine concentrations in healthy infants who had been suspected to have malabsorption, but who subsequently grew normally and had normal small intestinal biopsies, when compared with the concentrations in infants of the same age with untreated, later confirmed coeliac disease The one case (A D G 31) with a recognized gross mental disturbance showed initially very high piperidine levels in the urine (7 8 mmol/m creatinine) which decreased upon rehabilitation (3 2 mmol/m)

DISCUSSION

Our results for the urinary excretion of piperidine in the urine of adult men are less than those reported by von Euler (7), but very similar to more recent values obtained by gas chromatography (3 16) Theoretically variation in piperidine excretion could be due to several factors these include (*a*) substrate availability dependent on lysine intake, gut secretion and malabsorption (*b*) appropriate bacteria in adequate numbers in the gut (infection antibiotics), (*c*) gut transit time, (*d*) carbohydrate concentration and pH of the gut contents (pH optimum for *Escherichia coli* lysine decarboxylase=4 5 (9)), (*e*) availability of coenzyme (pyridoxal phosphate), and (*f*) tissue amine oxidase activity

The higher mean and great variation observed in adult women is of special interest. The possibility of variation with the menstrual cycle is at present being further investigated Sex differences in gut transit time could possibly be of importance in this context

The very low piperidine excretion in the newborn, as compared with older infants, indicates that this parameter could be a sensitive biochemical index of the postnatal bacterial colonization An analogy is the decreased concentrations of histamine in the gut lumen and gut wall of germ free mice as compared with conventional mice and the increased levels of histamine in the fecal lumen in germ free mice mono-infected with *Clostridium perfringens* (5) In contrast, other bacteria free characteristics, including cholesterol/coprostanol conversion, fecal mucin content and proteolytic activity of feces, change first at around one year of age (11)

The differences between breast-fed and formula fed full term and premature infants could be explained both by the increase in proteolytic bacteria on weaning (12) and the increased protein intake Heterocyclic amine excretion in the urine is known to increase during the first 48 hours after weaning in piglets (13, 21) Furthermore, a meat diet is known to increase the catabolic activity of the intestinal flora (15)

Dicarboxylases in bacteria are highly inducible enzymes (8) and the increased excretion of piperidine in the urine of formula fed infants does not necessarily imply a changed pattern of bacterial flora, or increase of the flora

The high excretion of piperidine of coeliac infants, as compared to infants without malabsorption, could be a consequence of the increased lysine content of the gut lumen and a direct parallel to the findings in cystinuria (19) The observed differences might also be a parallel to the phenol excretion in urine found in the "contaminated bowel syndrome" (16), or the increased excretion of tyramine found in a patient with severe malabsorption (22)

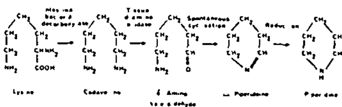


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Results are summarized in Table 1 Adult women show a higher mean than men, but

Table 1 *Urinary excretion of piperidine (mmol/m creatinine) in healthy infants adults and untreated coeliac disease of infants*

Age	*n*	Mean	S E M	*P*	Undetected in *n*
Premature infants					
2–6 weeks					
Breast fed	6	–	–		6
Formula fed	4	1 98	1 0		
Term infants					
0–6 days	17	0 29	0 09	0 001	10
1–2 months	9	1 93	0 30		1
4–6 months					
Breast fed	4	1 65	0 55	0 01	
Formula fed	4	5 95	0 56	0 005	
1–2 years	11	1 63	0 38		
Adult men	12	0 56	0 14		
Adult women	12	1 54	0 49		
Infants					
6 months–1 year					
No malabsorption	9	1 11	0 95	0 01	
Coeliac disease	5	3 62	1 40		

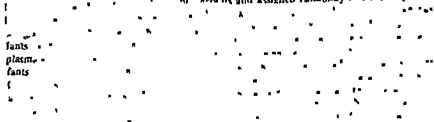
Acta Pædiatr Scand 69 647–652, 1980

THE METABOLIC CONSEQUENCES OF HUMAN MILK AND FORMULA FEEDING IN PREMATURE INFANTS

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From the Department of Paediatrics University Medical School Pecs Hungary

ABSTRACT Schultz, K , Soltesz, G and Mestyan, J (Department of Paediatrics, University Medical School Pecs Hungary) The metabolic consequences of human milk and formula feeding in premature infants Acta Paediatr Scand, 69 647, 1980 —Twenty premature low ... groups and assigned randomly to either a pooled



fants ...
plasma ...
fants ...

formula fed infants regained their birthweight more slowly than human ... protein formula feeding causes potentially unfavorable metabolic and amino acid imbalances in preterm infants in the early postnatal life

KEY WORDS *Preterm infants* nutrition milk proteins, plasma free amino acids, human milk, formula milk

Most term infants grow normally and do well if fed breast milk or a cow s milk based infant formula but there is still controversy among paediatricians over what should constitute the best milk for preterm infants The protein of cow s milk and of human milk differ qualitatively as well as quantitatively

Recent studies by Raiha et al (21) of the effects of varying the quantity and quality of milk protein on the growth and metabolism of preterm infants showed that there was no apparent growth advantage to a higher protein intake moreover the infants who were fed higher protein milk formula showed plasma amino acid imbalances azotaemia and metabolic acidosis which were not present in the breast milk fed infants

The metabolic consequences of different feeding practices are of particular importance because the developing central nervous system is sensitive to biochemical stress Since there are only few data on the biochemical effects of human and bovine milks in preterm newborn infants (2, 21), the aim of this study was to present some additional data on the metabolic effects of human milk and formula feeding in the early postnatal life of preterm infants

MATERIAL AND METHODS

Twenty low-birthweight infants were selected from the patients admitted to our premature ward All infants were physically normal on admission and had no signs of disease Birthweights of the infants fell between the tenth and ninetieth percentiles of the local intrauterine growth chart Mean birthweight gestational age and ranges are shown in Table 1 Ten infants were fed with pooled mature human milk and ten with the cow s milk protein based standard formula Robolact In all cases feeding was started before the age of 24 hours usually at the age of 6 to 12 hours The volume of the feeding was gradually increased and all infants received 170–180 ml/kg/day by the end of the first postnatal week

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Supported by the Scientific Research Council Ministry of Health Hungary (3 23-0502-04-1/M)

In addition to having a sedative and anti-aggressive effect, piperidine is known to be a strong emetic agent, to increase blood pressure and cause contraction in isolated ileum (7, 10) Recent work suggests that piperidine may act on cholinoceptive neurones as a modulator of synaptic activity The fact that endogenous piperidine multiplies by a factor of 18 during sleep in mice suggests that it is involved in the physiology of this state (10) In mammals, piperidine seems to have ready access to the central nervous system (14) and the present findings indicate that piperidine, and possibly other metabolites of gut bacterial origin, may induce the mental changes seen in untreated coeliac disease in infants

ACKNOWLEDGEMENT

This work was supported by Grant No 2583 from the Swedish Medical Research Council

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Submitted Aug 17 1979
Accepted May 12 1980

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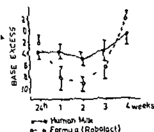


Fig 3 Effect of different feedings on base excess in blood

human milk than in the infants fed formula from the first week of life during the entire study period (Fig 2) There were no hypoglycaemic blood values observed during the study in the two groups of infants

Blood lactate and plasma free fatty acid concentrations did not change significantly

The concentration of alfa amino nitrogen varied, but was somewhat higher in the formula fed group, and this difference reached the level of significance at 24 hours and 3 weeks of age

Blood urea nitrogen was significantly higher in the infants fed by the formula The mean blood urea nitrogen concentration decreased after the second week of life but was still above the normal range during the entire period of study

Those infants receiving the high protein formula developed late metabolic acidosis during the second and third postnatal week, the infants on human milk had nearly normal base excess values (Fig 3) No significant differences in mean P_{CO_2} were found between the two groups of infants

Generally the concentrations of most free amino acids in plasma were higher in those infants fed the formula than in those infants fed human milk The total concentration of the 15 plasma free amino acids determined was significantly higher at the first day and at the second week of life in infants fed formula milk (Fig 4) The plasma concentration of aspartate in infants fed pooled human milks tended to be lower than in those infants fed formula milk The difference was significant at 2 and 4 weeks of age. In the first two weeks of the study the plasma concentrations of glycine and alanine were higher in the formula fed group The infants fed the high protein formula had plasma phenylalanine concentration two- to fourfold higher than those fed human milk The concentration of lysine in the plasma of infants fed formula milk was consistently higher than those of infants fed human milk The changes of the 15 individual plasma amino acids during the study period are summarized in Table 3

DISCUSSION

Postnatal weight gain in newborn infants is an important indicator of the nutritional status Snyderman et al (25) and Omans et al (17) found similar weight gain in premature infants receiving a wide range of protein intake (2 to 9 g/kg/day) In the present study we could not demonstrate a significant difference in the mean rates of weight gain, after regaining birthweight, between the two groups of infants Formula fed infants regained their birthweight later than human milk fed infants The poor weight gain observed in the first two weeks of life in preterm infants who were fed formula, might have been due partly to late metabolic acidosis and mild diarrhoea, which were frequently seen in these babies.

The data of the present study indicate that feeding preterm infants with high protein cow's milk based formulas (4 4 g protein/kg/day) has many metabolic disadvantages The fasting blood glucose was significantly lower

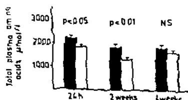


Fig 4 The effect of different feedings on the total concentration of 15 plasma amino acids in preterm infants (Black columns represent formula fed infants)

Table 1 *Clinical data of the infants studied*

	Formula (Robolact)	Human milk
Birthweight (g)	1 692 (1 320–1 960)	1 704 (1 450–1 920)
Gestational age (wk)	33 0 (30–36)	33 4 (30–37)
Number of infants	10	10

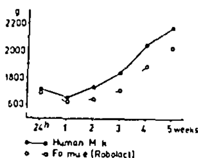


Fig 1 Mean postnatal weight gain in the infants studied

The composition of the formula and of human milk the daily intake of fluids protein and calories are compared in Table 2

Blood was drawn from peripheral vein at 24 hours of age and then at weekly intervals before the first morning feeding during the four week study period for the estimation of different metabolites Blood glucose (18), blood lactate (12) plasma alfa amino nitrogen (23) blood urea nitrogen (3) plasma free fatty acid (4) and acid-base status of the infants were measured weekly amino acid analysis was performed biweekly on blood samples by an automatic Beckman Multichrom Liquid Column Chromatograph The levels of 15 amino acids were quantitated The statistical significance of the data was tested using Student's *t* test

RESULTS

There were no feeding problems in any of the infants studied Six infants in the formula fed group developed mild diarrhoea during the first two weeks of life which lasted only for 1 to 3 days Repeated stool cultures were negative No diarrhoea occurred in the human milk fed group of infants

Table 2 *Composition of the formula compared to human milk and the daily intake of fluids calories and protein*

	Formula (Robolact)	Human milk
Protein g/100 ml	2 6	1 2
Fat g/100 ml	1 5	0
Carbohydrate g/100 ml	10 0[a]	7 0[b]
Fluid intake ml/kg/day	170	170
Calorie intake cal/kg/day	110	120
Protein intake g/kg/day	4 4	2 0

[a] Lactose 3 7 g/100 ml Saccharose 1 3 g/100 ml Dextrin maltose 5 0 g/100 ml
[b] Lactose 7 0 g/100 ml

There was no significant difference in the mean initial weight loss between the two groups of infants, but there was a difference in the mean time from birth to the time of regaining birthweight between formula fed infants (2 5 weeks) and human milk fed infants (1 5 weeks) (Fig 1) This difference was however not statistically significant After regaining birthweight there was no difference in the mean rates of gain in weight between the two groups during the study

The concentration of fasting blood glucose was significantly higher in the infants fed

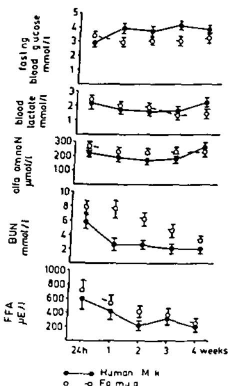


Fig 2 Metabolic responses to different feedings in premature infants

kg/day exceeds the needs of the preterm infants for protein synthesis The correlation between the higher BUN values and the protein intake of the premature infants has been observed previously (5 17 21)

The increased incidence of metabolic acidosis developing after the first week of life among formula fed preterm infants has been observed by several investigators (1 13 21 24) and has been related to the increased protein intake (27) The quality of protein also is of importance as it has been shown by different authors (2 14 21)

The preterm infant has a limited capacity for metabolism of amino acids because many of the enzymes involved are relatively inactive at birth (22) If the daily protein intake exceeds the capacity of the preterm infant for amino acid degradation amino acid clearance by the kidney or amino acid incorporation into protein hyperaminoacidaemia will develop High concentrations of free amino acids in the plasma of preterm infants fed high protein formulas have been found by several authors (10 15 19 20 26) The amino acids most often affected were tyrosine phenylalanine threonine valine leucine isoleucine methionine glycine in these studies In the present study the elevations of glycine alanine phenylalanine and lysine were striking in the formula group Similarly to the findings of Rassin et al (20) phenylalanine concentrations as high as those found in phenylketonuria were observed in a few infants fed the formula Hambraeus & Wranne (11) reported that premature infants who are fed human milk have a lower prevalence of hyperphenylalaninaemia than those fed cow s milk formula The clinical significance of these plasma amino acid elevations is still uncertain

Comparing the data of two feeding studies in premature infants in a recent review Davies & Evans (6) concluded that feeding human milk to these infants had produced a situation in many ways analogous to the effects of uteroplacental insufficiency Fomon et al (9) mean that the concentrations of protein calcium and sodium per unit of energy in human milk are insufficient for adequate growth of preterm infants in the immediate postnatal period

The question of what should constitute the best milk for preterm infants is a challenge to pediatricians, and still difficult to answer From the results of the present paper it can be concluded, that high protein formula feeding causes potentially unfavourable metabolic imbalance in preterm infants Human milk provides a safe nutritional management in the early postnatal life although further research is needed of how human milk should be supplemented for preterm infants

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Table 3. *The effect of different feedings (formula versus human milk) on plasma free amino acids in preterm infants*

Concentrations are given in μmol/l F=formula milk fed group H=human milk fed group

		24 h	2 weeks	4 weeks
Taurine	F	272±32	136±20*	84±12
	H	225±26	81±10	83±9
Aspartate	F	51±4	62±7**	61±5***
	H	46±6	39±4	43±3
Proline	F	187±23	134±10	141±24
	H	191±32	134±16	170±17
Glycine	F	308±23	254±22*	204±23
	H	277±9	166±16	206±10
Alanine	F	334±20	252±14***	263±39
	H	317±26	191±12	298±28
Cystine	F	144±33	91±10	65±11
	H	112±17	64±9	74±5
Valine	F	165±11	131±13	143±28
	H	124±16	127±17	152±22
Methionine	F	28±3	16±3	20±5
	H	22±7	13±1	23±1
Isoleucine	F	46±2	34±2	41±7
	H	42±7	38±5	47±6
Leucine	F	101±9*	82±6	84±10
	H	65±12	87±8	101±16
Tyrosine	F	129±19	76±11	85±26
	H	93±19	76±18	81±15
Phenylalanine	F	125±11***	249±74**	222±36***
	H	59±12	43±7	83±30
Lysine	F	206±22	283±55**	246±53**
	H	155±30	114±29	91±28
Ornithine	F	73±10	76±9	105±32
	H	110±36	48±11	51±9
Arginine	F	66±10	66±12	49±8
	H	52±12	41±7	76±6
Total	F	2 225±118*	1 877±156***	1 813±165
	H	1 863±121	1 283±52	1 606±121

$^*p<0.05$ $^{**}p<0.02$ $^{***}p<0.01$

in the formula fed group from the first week of life during the entire study period in spite of the higher carbohydrate intake in this group (10 g/100 ml versus 7 g/100 ml). The different carbohydrate composition of the two milks is very unlikely to account for the significant difference in the fasting blood glucose concentration, since the disaccharide-splitting enzymes are very efficient even in young infants (7) and except for the mild (probably fermentative) diarrhoea during the first 1 to 3 days no signs of deficient carbohydrate absorption were seen. The more likely explanation may lie the protein quality and especially amino aci composition of the feeds. Lindblad et al. (1(have shown that the content of branche chain amino acids is much higher in cow's mil protein than in brest milk protein. This differ ence could be of importance, as there are in dications that the branched chained aminc acid and tyrosine levels of plasma in particular influence insulin metabolism (8).

The prolonged azotaemia observed in our formula fed infants suggests that 4.4 g protein/

NET ABSORPTION OF STARCH IN LOW BIRTH WEIGHT INFANTS

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ABSTRACT Senterre, J (Department of Paediatrics, University Hospital, Liege, Belgium) Net absorption of starch in low birth weight infants Acta Paediatr Scand, 69 653, 1980.— T ... ere carried out in 12 low birth weight infants fed an



point of view, small amounts of starch in infant formulas ... thickener but also as a source of calories

KEY WORDS Starch, fat, nitrogen balance, absorption, low birth weight infants, infant formulas

Natural starch is a mixture of linear polymers (amylose) and of branched polymers (amylopectin) of D-glucose Starches from most plant sources from which it is refined contain about 18 to 27% amylose and the rest as amylopectin Two of the major exceptions are corn and rice starches which consist mainly of amylose Starches form part of many commercially prepared infant foods including infant formulas primarily because of various technical needs for making processed foods thicker and more uniform in consistency

Interest in starch digestibility in early infancy arises from two main concerns first to what extent starches can be considered as a caloric source and secondly what is the potential risk of undigested starches producing diarrheal symptoms and malabsorption Many factors are involved in starch digestion salivary alpha amylase activity gastric emptying rate pancreatic alpha amylase activity glucoamylase and maltase activities of the intestinal mucosa and glucose absorption (8, 9) It has been clearly demonstrated (2 5 6, 10, 14, 21) that in early infancy the potential rate limiting factor of starch digestion is the lack of pancreatic amylase which catalyses the random hydrolysis of amylose into maltotriose and maltose and that of amylopectin into branched dextrins However, clinical trials carried out in young infants by measuring either net absorption (4, 7), duodenal degradation products (3), or blood glucose (1, 11, 12) after starch feeding lead to different conclusions

The aim of the present study was to evaluate the nitrogen, fat and starch digestibility in low birth weight infants fed an infant formula containing 2% corn starch

SUBJECTS AND METHODS

Twelve 3 day balance studies were carried out with parental consent in 12 male low birth weight infants 1 to 4 weeks old Biometric and feeding characteristics of the infants are detailed in Table 1 An adapted formula with lactose as the sole carbohydrate was given to some of the infants as the initial feeding Then an infant formula containing 1 9 g/dl of pregelatinized corn starch was introduced at least 4 days before the beginning of stool collection Major constituents of the formula are protein 1 9 fat 3 5 lactose 4 6 sucrose 2.3 starch 1 9 and minerals 0 3 (all in g/dl) i e 74.3 kcal or 311 kJ/dl

During the balance period, intake of milk was measured by weighing of the bottle before and after each feeding. Separate collection of stools and urine was performed

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Submitted Aug 9 1979
Accepted Oct 16 1979

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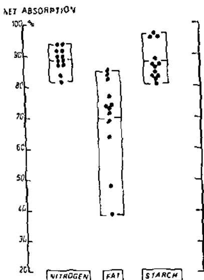


Fig 1 Net absorption coefficients of nitrogen fat and starch in low birth weight infants fed an infant formula containing 2% of corn starch

this method the recovery of a known amount of starch added to homogenized stools was 96–98%

Statistical analysis has been carried out with Student s *t* test and by regression analysis with determination of the p value

RESULTS

The individual results of starch, fat and nitrogen balance studies are detailed in Table 2 Mean starch intake was 3 55 g/kg/day and the net absorption coefficients ranged from 81 to 97% with a mean value of 88% Although starch absorption was not complete, no infant had diarrheal symptoms Number of stools per day was 4 4±0 6 and total stool weight was 14 7±4 2 g/kg of body weight per day Mean nitrogen intake was 584 mg/kg/day Mean fecal excretion of nitrogen was 61 mg/kg/day and mean nitrogen retention was 326 mg/kg/day These figures correspond to coefficients of net absorption and protein retention of 90% and 56% respectively The net fat absorption coefficients ranged from 39 to 85% with a mean value of 70% The two lowest coefficients were observed in infant No 1 who was the most premature at birth and in the infant No 12 who was small for-dates. The coefficients of net absorption of nitrogen, fat and starch are shown in Fig 1 Statistical analysis of the data did not show any correlation between the starch coefficients and the nitrogen or fat absorption coefficients. In addition, there was no relationship between starch absorption and the duration of starch feeding or the actual gestational age.

DISCUSSION

We observed that the coefficients of net absorption of starch ranged from 81 to 97% in low birth weight infants fed about 3 5 g of pregelatinized corn starch from an infant formula. As discussed by Filer (8) and DeVizia et al (7), the adequacy of fecal measurement of glucose and polysaccharide as indicators of starch digestion and absorption may be questionable since polysaccharide might be metabolized by gut microflora However, after the introduction of starch formula, the aspect and number, as well as the total weight of stools were not modified and there was no evidence of acid diarrhea

Although incomplete, the high digestibility of starch observed in low birth weight infants, contrasts with the very low pancreatic alpha-amylase activity reported by many authors in both resting (2, 5, 10, 21) and stimulated (6, 14) duodenal secretions in young infants After studying preterm and term infants, Zoppi et al (21) claimed that the early administration of small amounts of amylose increases the pancreatic alpha-amylase activity 3 to 10 fold However, their data indicate that even after 1 month of starch feeding, pancreatic alpha-amylase activity remains less than 2% of the average activity seen in older children In the present study no correlation was found between the coefficients of starch absorption and the duration of starch administration before the balance period As suggested by DeVizia et al (7), relatively high coefficients of starch absorption in infants may be explained by the fact that intestinal amylase

Table 1. *Biometric and feeding characteristics of the 12 infants*

No	At birth		During balance period		
	Weight (g)	Gest age (weeks)	Weight (g)	Age (days)	Prior starch feeding (days)
1	2 140	34	2 900	32	4
2	2 020	34	2 930	43	7
3	2 360	38	2 460	11	10
4	1 910	36	3 040	33	4
5	2 550	38	2 830	31	24
6	2 330	37	2 570	15	12
7	2 140	37	2 520	15	12
8	2 700	37	2 760	11	11
9	2 410	38	2 770	12	12
10	2 520	38	2 790	27	12
11	2 520	38	3 190	33	15
12	1 860	37	2 940	33	5
Mean	2 290	36 8	2 810	25	11
S D ±	270	1 5	210	11	6

with a metabolic bed as previously described (15) Each stool was immediately stored at −30°C until analysis For each infant, nitrogen fat and starch content of milk and stools were determined in duplicate In addition total urine nitrogen was measured over 3 days Total nitrogen was measured using the micro Kjeldahl technique and fat by Van de Kamer s method (19) Starch was assayed in milk and stool by determining glucose content before and after enzymatic hydrolysis according to a modification of the method described by Auricchio et al (4) The solubilization and hydrolysis of starch in the stools were performed by boiling an aliquot of 25 ml of stool homogenate at 100°C during 60 min After cooling 25 ml of acetate buffer containing 20 mg of amyloglucosidase from *Rhizopus delamar* was added and incubated overnight at 37°C After incubation the volume was brought to 100 ml with distilled water and 20 ml were deproteinized with 2 5 ml of 10% $ZnSO_4$ and 2 5 ml of 0 6N NaOH After centrifugation, glucose concentration in the supernatant was determined by the hexokinase method The starch content of the stools was calculated by multiplying the difference in glucose content measured before (free glucose) and after enzymatic hydrolysis of starch by 0 9 The factor of 0 9 is based on the fact that during hydrolysis water is added to the starch molecule with a consequent increase in weight of approximately 10% Using

Table 2 *Starch fat and nitrogen balance in 12 low birth weight infants fed an infant formula containing 2% of corn starch*

Infant no	Starch			Fat			Nitrogen			
	Intake (g/kg/d)	Stool (g/kg/d)	Absorption (%)	Intake (g/kg/d)	Stool (g/kg/d)	Absorption (%)	Intake (mg/kg/d)	Stool (mg/kg/d)	Urine (mg/kg/d)	Retention (mg/kg/d)
1	3 34	0 12	96	6 80	3 56	48	554	35	255	264
2	3 39	0 53	84	6 89	1 88	73	562	55	205	302
3	3 31	0 61	82	6 73	1 03	85	549	31	287	231
4	3 39	0 12	96	6 71	1 17	83	538	58	194	286
5	3 32	0 11	97	6 76	1 74	74	536	88	183	265
6	3 55	0 39	89	6 58	1 51	77	583	46	124	413
7	3 62	0 48	87	6 72	1 91	72	595	95	119	381
8	3 26	0 61	81	5 79	1 77	69	545	67	233	245
9	3 94	0 56	86	7 00	1 11	84	648	52	181	424
10	4 31	0 71	84	8 03	2 91	64	715	74	190	451
11	3 39	0 59	83	6 30	1 67	74	562	67	124	371
12	3 75	0 48	87	6 96	4 22	39	620	67	278	275
Total										
Mean	3 55	0 44	88	6 86	2 04	70	584	61	197	326
S D ±	0 31	0 21	6	0 41	1 00	14	54	19	58	77

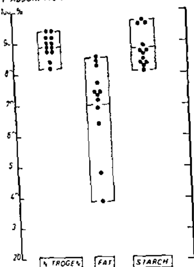


Fig 1 Net absorption coefficients of nitrogen fat and starch in low birth weight infants fed an infant formula containing 2% of corn starch

this method the recovery of a known amount of starch added to homogenized stools was 96–98%

Statistical analysis has been carried out with Student s *t* test and by regression analysis with determination of the *p* value

RESULTS

The individual results of starch fat and nitrogen balance studies are detailed in Table 2 Mean starch intake was 3 55 g/kg/day and the net absorption coefficients ranged from 81 to 97% with a mean value of 88% Although starch absorption was not complete no infant had diarrheal symptoms Number of stools per day was 4 4±0 6 and total stool weight was 14 7±4 2 g/kg of body weight per day Mean nitrogen intake was 584 mg/kg/day Mean fecal excretion of nitrogen was 61 mg/kg/day and mean nitrogen retention was 326 mg/kg/day These figures correspond to coefficients of net absorption and protein retention of 90% and 56% respectively The net fat absorption coefficients ranged from 39 to 85% with a mean value of 70% The two lowest coefficients were observed in infant No 1 who was the most premature at birth and in the infant No 12 who was small-for-dates The coefficients of net absorption of nitrogen, fat and starch are shown in Fig 1 Statistical analysis of the data did not show any correlation between the starch coefficients and the nitrogen or fat absorption coefficients In addition, there was no relationship between starch absorption and the duration of starch feeding or the actual gestational age

DISCUSSION

We observed that the coefficients of net absorption of starch ranged from 81 to 97% in low birth weight infants fed about 3 5 g of pregelatinized corn starch from an infant formula As discussed by Filer (8) and DeVizia et al (7), the adequacy of fecal measurement of glucose and polysaccharide as indicators of starch digestion and absorption may be questionable since polysaccharide might be metabolized by gut microflora However, after the introduction of starch formula, the aspect and number, as well as the total weight of stools were not modified and there was no evidence of acid diarrhea

Although incomplete, the high digestibility of starch observed in low birth weight infants, contrasts with the very low pancreatic alpha-amylase activity reported by many authors in both resting (2, 5 10, 21) and stimulated (6, 14) duodenal secretions in young infants After studying preterm and term infants, Zoppi et al (21) claimed that the early administration of small amounts of amylose increases the pancreatic alpha amylase activity 3 to 10 fold However, their data indicate that even after 1 month of starch feeding, pancreatic alpha-amylase activity remains less than 2% of the average activity seen in older children In the present study no correlation was found between the coefficients of starch absorption and the duration of starch administration before the balance period As suggested by DeVizia et al (7), relatively high coefficients of starch absorption in infants may be explained by the fact that intestinal amylase

activity of pancreatic or salivary origin, although low, is still sufficient to digest most dietary starch It may also be explained by the fact that mucosal glucoamylase (an enzyme that splits starch and dextrins into glucose) not only completes but also provides alternative pathways for intestinal digestion of starch. In this way, Kerzner et al. (13) recently showed in piglets without pancreatic amylase activity, that absorption of glucose polymers is 75% of the glucose equivalent

In infants 1 and 3 months old fed 2–3 5 g/kg/day of various natural starches, DeVizia et al (7) found coefficients of starch absorption greater than 98% in all infants studied However, an acid diarrhea was observed in 2 of 5 infants fed 6 g of starch/kg/day and they concluded that starch digestibility is limited in infancy Similar conclusions were drawn by Auricchio et al (3) who found incomplete intestinal hydrolysis of amylopectin in infants 6 months of age or younger Anderson et al (1), who have followed blood glucose concentration as an index of starch digestion and absorption observed a small but sustained rise of blood glucose in 3-day-old infants fed 2 g/kg of corn starch Thus these previous data and the present study suggest that the ability of young infants to digest large quantities of starch is limited, and that this is a result of low pancreatic alpha-amylase activity However, from a practical point of view our results show that, even in low birth weight infants, small amounts of starch in formulas can be considered not only as a thickener but also as a source of calories The fat malabsorption that we observed especially in the infant who was the most premature at birth and in the one who was small-for dates, may be explained by a lack of pancreatic lipase activity (14, 21), low bile salt excretion (20) and a high calcium and saturated fatty acid intake (16, 17) From our previous studies (18), it can be postulated that the supplement of energy provided as starch (about 12 kcal/kg/day) compensated for the fecal loss of fat and played a positive role in the nitrogen balance observed However, if starch has the advantage of increasing the caloric content of formulas without increasing their osmolarity, malto-dextrins having similar properties have been found to be completely digested (1, 9), and are probably more appropriate for increasing carbohydrate content of formulas destined for low birth weight infants

ACKNOWLEDGEMENTS

This research was supported by the Belgian Medical Research Fund Adapted formula (Pre Aptamil) and starch formula (Aptamil) were kindly provided by Milupa Friedrichsdorf Germany) The author thanks Mariette Cops for her skillful technical assistance

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Submitted Febr 19 1980
Accepted March 28 1980

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Acta Pædiatr Scand 69 659–662, 1980

CHOLIC ACID AND CHENODEOXYCHOLIC ACID CONCENTRATIONS IN SERUM DURING INFANCY AND CHILDHOOD

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ABSTRACT Heikura, S., Simila, S., Finni, K., Maentausta, O. and Janne, O. (Departments [illegible] Finland) [illegible] and childh[illegible] [illegible] or the peripheral vein serum of adults. The levels of cholic and chenodeoxycholic acid remained high until the age of 6 months, being about 5-fold higher than those in the sera of adults. Primary bile acid concentrations reached the adult level by the age of 1–2 years. These results indicate that developmental changes occur in the metabolism and excretion of bile acids in man. The relatively high concentrations of the primary bile acids in serum during the first 6 months of life suggest that up to this age, the mature ability of the liver to excrete the bile salts into the bile and/or to clear them from the circulation has not yet been reached.

KEY WORDS: Cholic acid, chenodenoxycholic acid, bile acids, infants, children, liver function

Since the introduction of radioimmunoassay for bile acid measurement (19), a great deal of information has been obtained about serum bile salts and their changes in adults in connection with various physiological and pathological conditions (2, 4, 5, 6, 8, 10, 15, 20), but corresponding knowledge concerning infants and children is largely lacking (7, 12, 17, 21). Bile acid synthesis, pool size, biliary secretion and urinary excretion in infants are different from those in adults (7, 12, 17, 21), but whether a low biliary secretion of bile acids during infancy (1, 3, 14) is due to immaturity of liver function is not known. Changes in the activity of serum γ-glutamyl transpeptidase during the early months of life suggest the presence of a cholestatic condition in the liver (16), which could, in turn, explain the diminished bile salt secretion at this stage.

The aim of the present work was to delineate physiological changes in serum bile acids during infancy and childhood by measuring, with radioimmunoassay, the concentrations of the two primary bile acids (cholic and chenodeoxycholic acid) from sera of healthy children from one hour to 15 years of age. The results obtained indicate that serum bile acid concentrations during the early months after birth clearly differ from those present in adults, and hence, when bile salt levels are used for evaluation of liver function in infants and children, age-matched reference values are mandatory for proper conclusions to be drawn.

MATERIALS AND METHODS

Blood samples

Samples were taken from the umbilical cord, and from the peripheral vein of infants and children at the ages of 1 hour, 1, 2 and 7 days, 1, 3 and 6 months, and 1, 2, 3–5, 6–12 and 13–15 years. The groups comprised 8–21 subjects, as indicated in detail in Table 1. Blood samples were obtained by venipuncture with permission of the

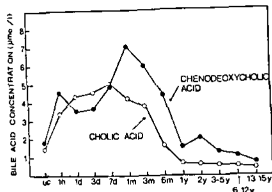


Fig 1 Changes in the mean concentrations of cholic acid and chenodeoxycholic acid in the serum of infants and children at different ages Each dot represents the mean value for the number of subjects given in Table I Abbreviations *uc* Umbilical cord *h* hour *d* day(s) *w* week(s) *m* month(s) and *y* year(s)

that there is a maturation process in hepatic bile acid metabolism and secretion which is completed within the first 1–2 years after birth

DISCUSSION

The bile acid metabolism of infants differs in a number of respects from that of adults (7 14 17 21) Firstly the rate of bile acid synthesis and the size of the bile salt pool are smaller than those in adults (21) Secondly bile acids are predominantly conjugated with taurine during infancy in contrast to adults, whose major bile acid derivates are glycine conjugated (1 3 4 12 14) Thirdly the intraluminal bile acid concentrations in the duodenum during meals are lower in newborns than in adults (1 3 13) a finding that may be related to the diminished ability of the gallbladder to concentrate bile salts during the early months of life (7 17 21) On the other hand the low intraluminal bile acid content may equally be derived from a poor hepatic secretion of bile acids into the bile thus leading to a preferential distribution of the bile acids to the peripheral circulation These immature characteristics of bile acid metabolism are reported to usually mature by the age of two years (7 17 21) In accordance with these findings, the data of the present work show that during the first year of life, primary bile acid concentrations in the serum are markedly higher than those in adults, the latter levels being reached by the age of 1–2 years In a previous study (18), Sandberg, who used gas liquid chromatographic techniques for bile acid measurement and a limited number of samples, did not find significant differences in serum bile salt levels between children and adults Whether the disparity between the present and previous results is due to methodological reasons remains to be elucidated

For obvious reasons, all the samples we studied were not taken after an overnight fast, but only 4 hours postprandially This should not lead to higher serum bile acid concentrations, since, at least in adults, meal induced elevations in the serum levels of cholic and chenodeoxycholic acid are reported to have subsided by 4 hours postprandially (5) Further, the intraluminal bile acid concentration in infants and children is low after meals, which makes it less likely that the shorter fasting period has led to the high serum bile acid values

Bile acids are mainly conjugated with taurine during infancy (7, 17, 21), while glycine-conjugates predominate in adults (see e g 4) The antisera used in this study for determination of the primary bile acids did not differentiate between taurine- and glycine conjugates of the bile acids, but recognized both types of conjugates equally well (10) It is, therefore, not likely that the high concentrations of cholic and chenodeoxycholic acid in the serum of infants and children are due to methodological reasons, i e a preferential measurement of taurine-conjugated bile salts

A high serum bile acid concentration is considered to indicate inefficient hepatic function, either due to parenchymal cell lesion or to cholestasis (4, 11, 15, 20) In addition to elevated serum bile acid levels and low postprandial intraluminal bile salt concentrations in the gut (1, 3, 13, 14), developmental changes

Table 1. *Concentrations of cholic acid and chenodeoxycholic acid and their ratios in the serum of infants and children*

The values are expressed as μmol/liter and are means ± S D for the number of samples in parentheses

Group		Cholic acid	Chenodeoxycholic acid	Ratio
Umbilical cord	(11)	1 43±0 41[c]	1 82±0 48[c]	0 87±0 27
1 hour	(10)	3 40±1 33[a,c]	4 59±1 92[a,c]	0 87±0 31
1 day	(16)	4 32±2 20[a,c]	3 55±2 45[a,c]	1 61±1 18
3 days	(14)	4 51±2 76[a,c]	3 67±2 00[a,c]	1 95±0 66
7 days	(10)	5 08±2 80[a,c]	4 83±1 82[a,c]	1 02±0 34[b]
1 month	(10)	4 18±2 31[a,c]	7 09±3 01[a,c]	0 73±0 22[b]
3 months	(10)	3 73±2 90[a,c]	5 99±2 59[a,c]	0 60±0 42
6 months	(9)	1 64±0 56[b,c]	4 40±1 26[a,c]	0 51±0 22
1 year	(10)	0 69±0 61[b]	1 54±1 42[b]	0 64±0 39
2 years	(8)	0 58±0 50	2 08±1 72	0 38±0 32
3–5 years	(19)	0 60±0 67	1 37±1 73	0 75±1 01
6–12 years	(21)	0 51±0 47	1 14±1 38	0 81±0 56
13–15 years	(20)	0 35±0 24	0 71±0 74	0 60±0 58

a

b ...pendent) than the value in umbilical cord serum

c ... p

... years

mothers serum was separated by centrifugation and stored at −20°C until assayed for bile acids Up to the age of 7 days a 4 hour fasting period preceded blood sampling and after this age blood samples were taken after an overnight fast

Determination of bile acids in serum

Cholic acid and chenodeoxycholic acid concentrations were separately measured from the serum as previously described (6 10) In short the radioimmunological techniques involved the following steps (*a*) extraction of 0 1 ml of serum with 0 9 ml of absolute ethanol (*b*) centrifugation of the precipitated proteins and evaporation of different portions (0 05–0 2 ml) of the ethanolic supernate in disposable glass tubes (*c*) redissolving of the residue in 0 01 M phosphate buffer (pH 7 4) and incubation of the samples with appropriate antisera and [^{125}I]iodohistaminyl derivatives of the bile acids at 4°C for 14–16 hours (*d*) precipitation of the bound radioactivity with polyethylene glycol (final concentration 12 5%) and (*e*) counting of the precipitate in a gamma counter Calculation of the results was conducted with the aid of standard curves run in parallel with each series of analytical samples The properties of the antisera employed (10) permitted specific measurement of the two bile acids The cross reactivity of the antisera with conjugates of a given bile acid did not however allow separate measurement of free bile acids and their glycine and taurine conjugates (10) rather each assay yielded values representing the sum of the free and conjugated bile acid concentrations

RESULTS

Table 1 shows the concentrations of cholic acid and chenodeoxycholic acid and their ratios in the various groups of samples studied Alterations in the mean serum concentrations of the primary bile acids with advancing age are illustrated in Fig 1 The concentrations of the two bile acids in umbilical cord sera (cholic acid 1 43±0 41 μmol/l and chenodeoxycholic acid 1 82±0 48 μmol/l) were significantly higher than those we have previously measured from the serum of pregnant or non-pregnant women with this technique (6, 10) The reason for this remains to be elucidated It is significant, however, that concentrations of the two primary bile acids were clearly higher in the infant serum than in the cord blood serum (Table 1 and Fig 1) When compared with the levels present in adult serum, primary bile acid concentrations were some 5–10-fold higher in the infant serum (cf Table 1 and refs 6, 10) The concentrations of primary bile acids declined to adult levels by the age of 1–2 years, the concentration of cholic acid reaching these levels somewhat earlier than that of chenodeoxycholic acid The latter bile acid was the predominant bile salt in the sera of children from the age of 1 month onwards, the mean cholic to chenodeoxycholic acid ratio ranging from 0 38 to 0 8 (Table 1) Taken together, these data suggest

Acta Pædiatr Scand 69 663–666, 1980

RELAPSE RATE AFTER CESSATION OF THERAPY IN CHILDHOOD LEUKEMIA

A Follow up Report on 277 Cases from the Five Nordic Countries

O J JOHANSEN and P J MOE

From the Department of Paediatrics University of Trondheim Trondheim Norway

ABSTRACT Johansen O J & Moe P J (Department of Paediatrics, University of Trondheim, Norway) Relapse rate after cessation of therapy in childhood leukemia A follow up report on 277 cases from the five Nordic countries Acta Paediatr Scand, 69 663, 1980 —Two hundred and seventy seven children from the Nordic countries who had their antileukemic therapy stopped before January 1979 were surveyed The children were in remission when therapy was discontinued So far 64 (23 1%) have relapsed Central nervous system (CNS) and testicular leukemia have been a problem, but CNS-prophylaxis has been in common use in the Nordic countries only since 1972–1973 Most of the patients relapsed during the first year after stopping therapy, whereas no patient relapsed later than 4 years after cessation of therapy

KEY WORDS Childhood leukemia cessation of therapy, Nordic countries, relapse rate

Only a few years ago childhood leukemia was considered to be incurable Because of encouraging preliminary studies, therapeutic programs became increasingly intensive by combining corticosteroids with several types of cytostatic agents Central nervous system prophylaxis and high dose methotrexate are the most recent advances in modern antileukemic therapy (6, 7 8)

A few years ago palliation and prolongation of life was the aim of therapy and patients in remission were considered to need life long supporting therapy It is now usual to discontinue therapy in children in remission for 2½–3 years (1 5) Indeed total therapy has altered the prognosis so that permanent cure is now a realistic goal in the treatment of acute lymphocytic leukemia

This report is in part a follow up of a report presented on 160 Nordic children who had their therapy discontinued prior to November 1976 (5) In this report data on all cases of childhood leukemia in the Nordic countries in whom therapy was stopped before January 1979 was collected and the effects of stopping therapy were evaluated

MATERIAL AND METHODS

The combined population of the Nordic countries Denmark Finland Iceland Norway and Sweden is about 22 million inhabitants Some 200 new cases of leukemia are diagnosed each year in individuals below 15 years of age

All 132 departments of paediatrics and 4 other departments treating children with leukemia in the Nordic countries were asked the following questions

1 Have you discontinued antileukemic therapy before January 1979 in any child in prolonged remission? Please give birth dates sex time of diagnosis and time of cessation of therapy

2 Have any of the cases relapsed? Please give time and site of relapse

Replies were received from all 136 departments that were surveyed in the 5 Nordic countries Several patients were reported from two departments but their reports were unified Several different therapeutic programs were used partly because the survey covered 17 years and because 5 different countries were involved It was beyond the scope of this study to collect information on the different treatment regimens or to evaluate them A high dose methotrexate regimen has only been in use during the last 4 years particularly in Norway (6 8)

RESULTS

In a total of 277 patients with acute leukemia, a prolonged remission had occurred and

Dedicated to prof Rolf Zetterstrom on the occasion of his 60th anniversary

in the activity of serum γ-glutamyl transpeptidase (16) and in the elimination rate of bromsulftalein (9) suggest that there might be a relatively cholestatic condition in the infant liver, which matures during the first years of life The high serum bile acid values during infancy may therefore be a reflection of two factors an increased rate of bile acid synthesis at this age (21), and the immaturity of the liver in secreting bile acids via the gallbladder to the gut

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Submitted Febr 18 1980
Accepted March 24 1980

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Acta Pædiatr Scand 69 663–666, 1980

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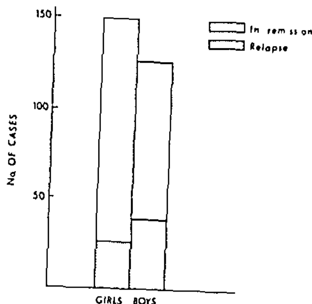


Fig 1 Sex distribution in the material

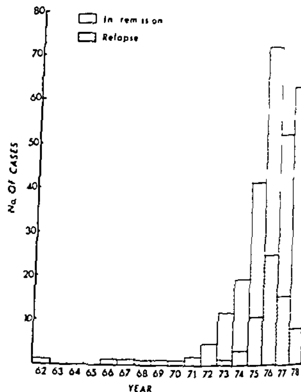


Fig 2 Year of cessation of therapy

therapy had been discontinued before January 1979 Fig 1 shows the sex distribution in the material 267 were reported as acute lymphocytic leukemia, 9 as acute myelogenous leukemia and 1 as promyelocytic leukemia Table 1 shows the number of cases off therapy, the number of cases in complete continuous remission, and number of relapsers in the individual Nordic countries

In only 24 cases diagnosed before 1968 had therapy been discontinued From 1968 there has been a marked increase in the number of cases off therapy About 25% of the cases diagnosed in 1973 (57 cases) had their therapy stopped before January, 1979

Fig 2 shows the year of cessation of therapy Only 8 children had therapy discontinued before 1972 64 of the patients relapsed before January 1979 The observation period for the patients who discontinued therapy in 1978 is short and several of them are expected to relapse during 1979 Table 2 shows the site of relapse 44 of the 64 relapsed during the first year, 16 during the second year, 3 during the third year and 1 during the fourth year (Fig 3) The relapse rate was 16 3% in the first year

Table 1 *Survey of the materials from the five Nordic countries*

Country	No of cases off therapy	Continuous complete remission	Relapse after cessation	% relapse
Denmark	46	33	13	28 3
Finland	63	54	9	14 3
Iceland	1	1	0	
Norway	69	55	14	20 3
Sweden	98	70	28	28 6
Total	277	213	64	23 1

The duration of therapy in the 277 patients is shown in Table 3 Three years on treatment was most common The relapse rates were quite similar for the different durations of therapy Two of the nine patients reported as acute myelogenous leukemia and the patient with acute promyelocytic leukemia relapsed Nine of the 277 patients were in their second remission when therapy was discontinued, only one relapsed 160 patients had therapy discontinued prior to November 1976 A total of 42 of the 160 patients had relapsed before January 1979, giving a relapse rate of 26 3% The observation time for the 160 patients was at least 26 months

Table 2 *Site of relapse*

Bone marrow	36
Central nervous system	6
Testis	14
Marrow+CNS	4
Marrow+testis	2
Other organs	2
Total	64

DISCUSSION

The survey of all childhood cases of acute leukemia in whom therapy was discontinued in the Nordic countries has been updated A total of 277 cases had antileukemic therapy discontinued prior to January 1979 No similar material from a similarly large geographic area has been published

About 25% of all 194 cases diagnosed as childhood leukemia in the 5 countries in 1973 had antileukemic therapy stopped George et al (1) reported the discontinuation of therapy in 278 cases of acute lymphocytic leukemia in St Jude Children s Research Hospital one of the principal centers for the treatment of childhood leukemia 235 of their 639 (36%) cases stopped therapy when they were in their first remission compared to 56 of 150 cases (about 35%) diagnosed as acute lymphocytic leukemia in the Nordic countries in 1973

After 1972 an increasing number had therapy stopped This was possible because the treatment regimens became more intensive and more patients attained remissions

In 1976 73 patients had therapy stopped These figures are very encouraging and indicate a breakthrough in leukemia treatment in the Nordic countries Forty patients were in remission for 4 years or more after stopping therapy So far no patients have relapsed after four years of discontinued therapy These data resemble those reported by George et al (1)

The relapse rate ranged from 14 3% to 28 6% in the Nordic survey (a mean of 23 1%) compared to about 20% at St Jude Hospital More aggressive therapy and CNS prophylaxis for a longer period of time have been used at St Jude Hospital than in the Nordic countries

The number of cases off therapy in relation to the expected diagnosed cases is somewhat lower in Denmark than in the other countries This is partly due to the fact that in Denmark there has been more reluctance to stop therapy after 3 years of prolonged remission than in the other Nordic countries (5) The relapse rate was highest in Sweden (28 6%) and lowest in Finland (14 3%)

The number of cases off therapy in each group was, however, small and the difference between some of the countries may therefore not be significant

The following conclusions may be drawn from these studies

1 The ultimate relapse rate after cessation of therapy will probably be between 25–30%

2 There was only a small difference in relapse rate between a large childhood cancer center and the 5 Nordic countries after cessation of therapy The observation time was shorter for some of our patients, but it must be borne in mind that the material represents 5 countries and a number of different regimens

3 The relapse rate was highest during the first year after cessation and decreased for

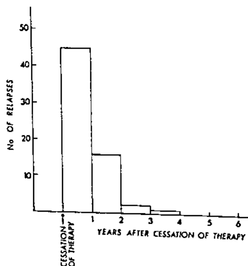


Fig 3 Yearly no of relapses after cessation of therapy

Table 3 *Duration of therapy*
Nine patients in secondary remission not included

Duration of therapy (years)	In remission	Relapse	% relapse
<3	13	4	23 5
3	82	27	24 8
3–4	47	12	20 3
4–5	21	7	25 0
>5	41	14	25 5

every year after cessation of therapy No relapse was observed after 4 years or more off therapy in a total of 118 cases in the two surveys

4 The relapse rate was higher in boys than in girls after cessation of therapy (Fig 1) Age did not seem to play a role in the relapse rate From the St Jude study it was concluded that other risk factors as initial leukocyte count, capability of lymphoblasts to form rosettes with sheep erythrocytes and the presence or absence of a mediastinal mass were of no value in predicting the length of complete remission after cessation of therapy (1)

Permanent cure is the ultimate goal of treatment of acute lymphocytic leukemia of children This possibility seems more likely since leukemia free survival after cessation of therapy has improved markedly The optimal duration of therapy is not known and the hazards of long term therapy must be weighed against the potential benefit (2, 3 4) Earlier studies indicate that the relapse rate was higher if the therapy was extended to more than 3 years (5) George et al found a higher relapse rate during the year before planned cessation of therapy than during the first year after cessation of therapy (1) Our study also indicates that maintenance therapy for more than 3 years is not justified in most cases (Table 3) The patients that possibly will benefit from longer maintenance therapy are those patients who have relapsed or will relapse, but these individuals cannot be identified at the time of cessation of therapy George et al states that 4 years of remission after cessation of therapy is a reasonable operational definition of cure Thus, 40 patients from the Nordic countries who were off therapy for 4 years or more may be considered cured from their leukemia, and the number may triple by 1981 Perhaps, by that time the risks of long term antileukemic therapy will become more evident We are particularly concerned whether late effects of intensive antileukemic therapy will surface Such information is of course crucial in the assessment of antileukemic treatment programs Data are being collected currently on intellectual capacity, sexual maturation, progeny and the development of secondary malignancy

ACKNOWLEDGEMENTS

cancer registers [illegible]
on new cases of childhood leukemia Financial support was obtained from the Norwegian Cancer Society

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Submitted Nov 5 1979
Accepted Febr 1 1980

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Acta Pædiatr Scand 69 667–673, 1980

ARTHROPATHY IN CHILDREN WITH SEVERE HEMOPHILIA A

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ABSTRACT Soreff, J and Blomback, M (Departments of Orthopaedic Surgery and Blood Coagulation Disorders, Stockholm, Sweden) Arthropathy in children with severe hemophilia A Acta Paediatr Scand, 69 667, 1980 —Seven children with severe hemophilia A were studied The radiological findings show that the patients developed joint changes despite our prophylactic treatment even if no restriction of movement or deformity could be detected by physical examination In contrast to earlier reports indicating that changes in the knee joints were most common, our findings show that changes in the ankle joints predominate Such changes developed, even after less than 10 registered joint bleeding episodes Prophylactic treatment must start early and should aim to eliminate subclinical bleedings also Increased dosage and shorter intervals between treatments lead to less bleedings The dosage must be so high and the time interval so short that the F VIII level in plasma is >0 01 U/ml at the time of the next infusion Prophylactic treatment must be regular and should be given even if bleeding requiring treatment with a therapeutic dose occurred in the previous day

The use of factor concentrate has altered the outlook for hemophiliacs and enables them to lead an almost normal life It is of interest, therefore, to evaluate the results obtained with this type of treatment with regard to arthropathy

PATIENTS AND METHODS

Seven patients aged 6–12 years (mean age 9 years) comprising all children in the Stockholm area treated by us for severe hemophilia A (Factor VIII deficient Factor

... was treated and if four days or more had elapsed between each treatment episode Clinical examination was performed to record any deformities and restricted joint movement The roentgenological assessment is based on the following criteria

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(Fig 2) ... ght joint incongruity

Grade III Marked cartilage destruction, marked deformities such as varus or valgus angulation or subluxation marked rotational deformity and joint incongruity (Fig 3)

Grade IV Advanced joint destruction with severe deformities subchondral bone destruction and total destruction of the cartilage (Fig 4)

Hematrate (=Hemophil) Prophylactic treatment was started in 2 patients at the age of 3 years in 2 others at the age of 5 years and in 3 patients at the age of 6 years In 3 patients prophylactic treatment was interrupted for some time

RESULTS

Bleeding frequency and the results of radiological and physical examinations are presented in Table 1 As can be concluded from Table 1, the most marked and frequent changes are seen in the ankle joints in our patients A preliminary report of this finding was presented in 1978 (8)

Even patient 7, who received early and consistent prophylactic treatment at home, showed Grade I–II ankle joint changes He had a few bleeds before the start of prophy-

Table 1. *Clinical and radiological changes in the joints of 7 hemophilic children in relation to total number of bleeding episodes*

Age=age at time of follow up when total number of bleeds were counted (late 1977) X ray follow up was made early in 1978 ( ) indicates episodes of bleeding into hands or feet which could have involved the joint on some occasions
* denotes slightly restricted movement

Joint	Pat 1 12 years			Pat 2, 10½ years			Pat 3 9 years		
	No of bleeds	Clinic chang [a]	Radiol chang	No of bleeds	Clinic chang	Radiol chang [b]	No of bleeds	Clinic chang	Radiol chang [c]
Shoulder									
Right	0	0	–	3	0	–	0	0	–
Left	0	0	–	1	0	–	0	0	–
Elbow									
Right	1	0	0	4	0	–	1	0	0
Left	1	0	0	16	0	–	20	Ext def 20°+deform	III
Wrist									
Right	2 (2)	0	–	0 (4)	0	–	0	0	–
Left	2 (2)	0	–	0	0	–	0	0	–
Hip									
Right	0	0	–	8	0	–	1	0	–
Left	0	0	–	2	0	–	0	0	–
Knee									
Right	10	0	–	7	0	I	1	[e]	II
Left	12	0	–	13	0	I	13	[f]	II III
Ankle									
Right	50 (9)	*	II–III	20	0	II–III	0 (3)	0	I
Left	40 (9)	*	II–III	20	Equinus 45°	II–III	2 (9)[g]	0	I

f femur [c] Left leg 1 5 cm longer [d] 2 cm shortening of left le
enu valgum 20° f lexion contracture 15° [g] In addition several bleeds

laxis During the 3 years before the present investigation no bleeding was recorded

Patient 3 shows only Grade I changes in the ankles but severe changes in the knees This patient had a congenital ventricular septal defect and was operated on at the age of 7 years Furthermore the parents failed to understand the importance of close contact with doctors and physiotherapists The patient was often carried by his parents when he bled into the knee joints and several bleeds were probably not treated Due to his heart disease and immobility during bleeding into the knee joints he probably did not run about as much as other children

Restricted movement in both feet was noted in patient 1, in whom prophylactic treatment was interrupted for some time because we suspected that a Factor VIII inhibitor was developing However, the titer of the inhibitor was low and treatment was started again In patients 2 and 5 prophylactic treatment was also interrupted, mainly due to difficulties in venipuncture (Table 2) Only patient 6 complained of pain (in the left ankle) All the other patients reported freedom from pain except when bleeding occurred

Three patients (nos 2 3 and 6) showed inequality of leg length in one case (no 2) due to a congenital defect

Except for one patient who had frequent nose bleeds like his mother, bleedings other than into joints were infrequent during prophylactic treatment

Despite a number of bleeding episodes, the patients did not have to interrupt their school

t 4 8½ years			Pat 5 8½ years			Pat 6 7 years			Pat 7 5 years		
o of eeds	Clinic chang	Radiol chang	No of bleeds	Clinic chang	Radiol chang	No of bleeds	Clinic chang	Radiol chang [a]	No of bleeds	Clinic chang	Radiol chang
[illegible]	0	–	1	0	0	2	0	–	0	0	–
[illegible]	0	–	0	0	0	1	0	–	0	0	–
[illegible]	0		50	Ext def 15°	II	2	0	–	3	0	–
0	0	–	2	0	0	0	0	–	1	0	–
[illegible]	0	–	0	0	–	1 (2)	0	–	1 (2)	0	–
[illegible]	0	–	0 (1)	0	–	0 (3)	0	–	0 (6)	0	–
0	0	–	3	0	–	0	0	–	0	0	–
1	0	–	2	0	–	0	0	–	0	0	–
4	0	I	1–2	0	I	2	0	0?	5	0	I?
3	0	–	2 3	0	I	3	0	0?	3	0	I?
8 (8)	0	I II	15 (12)	0	III	14 (2)	*	II	3 (3)	0	I–II
11 (32)	0	II–III	12 (13)	0	III	23 (5)	0 Pains	II	2 (1)	0	I–II

ing for more than a few days each year if at all

Tables 2 and 3 present some facts about the treatment with concentrates It should be mentioned that the incidence of bleeding is probably higher than noted as the patients did not always visit the hospital during bleeding episodes However bleedings noted after the start of home treatment are carefully registered The patients who show the least marked joint changes (1 4 5 6 and 7) have been looked after by the same physiotherapist

Gold injections into joints were associated with reduced bleeding frequency However, the prophylactic dosage was often increased at the same time

As is apparent from Tables 2 and 3 some of the parents and doctors were not sufficiently aware of the necessity for continuing regular prophylactic treatment even if a bleeding episode had just occurred and had been treated

When the level of factor VIII was about 0 3 U/ml in plasma after infusion, and when the interval between infusions was so short that a level of >0 01 U/ml was maintained until the next infusion, joint bleedings were rare or absent

DISCUSSION

So far arthropathy remains an inevitable complication of repeated joint bleeding and often causes severe disability in hemophilia A child of about 10 years needs Factor VIII concentrates from about 200 blood donors each week for adequate prophylaxis and the costs

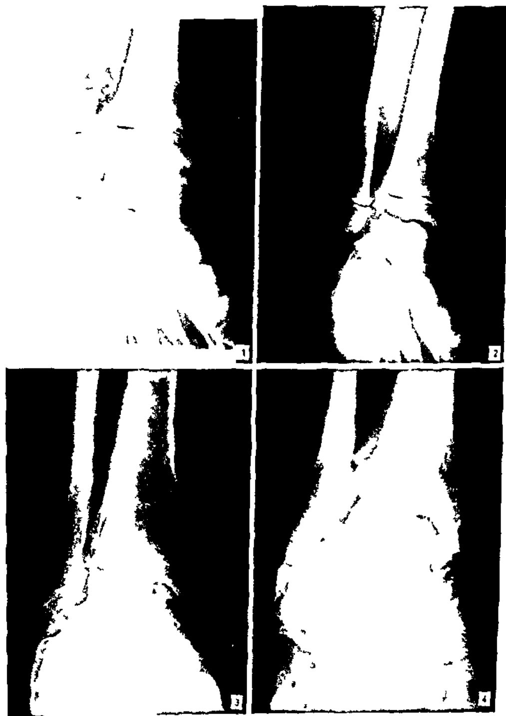


Fig 1 Hemophilic arthropathy Grade I Increased density of capsule and synovia Widening of the epiphysis and erosion of the cartilage at the joint margin
Fig 2 Hemophilic arthropathy Grade II Narrowing of the joint space irregular subchondral bone and subchondral cysts Very slight valgus deformity
Fig 3 Hemophilic arthropathy Grade III Pronounced cartilage destruction advanced changes in the subchondral bone marked valgus deformity
Fig 4 Hemophilic arthropathy Grade IV Total destruction of cartilage and subchondral bone severe joint incongruity and valgus deformity

for such treatment are enormous In addition we cannot exclude the risk of severe side effects of the treatment with concentrates such as liver damage and developing of circulating inhibitors which will deprive the patient of necessary treatment on many occasions Therefore every attempt to tackle these problems by further research must be encouraged

The development of joint changes depends on the degree of hemophilia the patient s age the patient s personality (4) and treatment of course also plays an essential part In addition some patients seem to have more severe joint changes than others even though the frequency of joint bleeding and treatment are the same It is possible that even within the group with severe hemophilia we are faced with different types of patients

It has been shown that hemophilic arthro

able 2 *Effect of different dosage regimens on frequency of joint bleeding in patients 1–4*

at o	Age[a] (y)	Type of F VIII conc	Frequency of infusion	F VIII (units/kg)	Level F VIII (U/ml plasma post inf)	Effect on joint bleeds
	5–6	Kabi	1/2nd w	10	–	–
	7–8	Kabi	1/w	8	0 27	–
	8–10	–	–	0	–	Circul anticoag suspect Ankle bleeds 10/month[e]
	10–11	Hematrate	2/w	17	0 22–0 35	High frequency in ankle
	11–12	Hematrate	2/w	–	–	High frequency in ankle
	12–13	Hematrate	Ev 3rd day	16	0 32	Ankle bleeds 1–2/month
	13–14	Hematrate	3/w	20	0 31	None in 5 months
2	6–7	Kabi	1/w	30–26	0 2/0 4/0 6	Frequent bleeds No definite effect of prophylaxis[b, a]
	7–8 5	–	–	0	–	Difficult venipuncture
	8 5–10	Hematrate	1/w	35–30	0 40	1–4/month
	11–12	Hematrate	2/w	25	0 29	2/8 months
3	6	Hematrate	1/w	28	0 41	Few recorded[c]
	7	Hematrate	1/w	22	0 32	Frequent bleeds between prophylactic inf esp left elbow, right foot and left knee[b, c]
	8–9.5	Hematrate	1/w	18	0 20	Frequent bleeds between prophylactic inf , esp left elbow, right foot and left knee[b, c]
	9 5–10 5	Hematrate	2/w	22	0 22	Frequent bleeds between prophylactic inf , esp left elbow, right foot and left knee[b, a]
	10.5–11	Hematrate	3/w	28	–	Still 1–3 bleeds/month, but much better than earlier
4	6–6 5	Hematrate	1/2nd w	20	0 28	1 bleed left ankle/month
	6 5–7 5	Hematrate	1/w	26	–	2–3 bleeds left ankle/month + some other bleeds
	7.5–8 5	Hematrate	2/w	27	–	Reduced bleeding freq
	8 5–10	Hematrate	2/w	30	0 26	2 left ankle/year

…n when treated for bleedings on previous
…eeds into right ankle after 5 yrs—reduced

pathy predominantly affects the knee, elbow and ankle joints (1, 4) The hip and shoulder joints seemed to be less susceptible, owing to their spheroidal construction with a broad joint surface which is less liable to get injured These joints also lack synovial recesses with get easily jammed

There is conclusive evidence that the development and severity of joint changes is related to a great extent to the severity of the hemophilia Thus patients with moderate hemophilia (1–4%=0 01–0 04 U/ml factor VIII) have few joint bleeds compared with severely affected patients (<1% factor VIII) Prophylactic treatment of patients with severe hemophilia (6) was first attempted in Sweden in the late 1950's This treatment aimed at providing a sustained rise in the level of factor VIII and in this way transform severe hemophilia into its moderate form The factor VIII concentrate initially used in 1956–1966, was prepared from fresh blood and on small scale (2) This preparation had an activity of 0 5–0 8 F VIII U/ml and if the level in the patient was increased to 0 25 U/ml, traces of factor VIII activity were still present some weeks after infusion (6) The prophylactic treatment of children reported in our study aimed at the same effect, but as the in vivo half life of the factor VIII concentrate used today is much shorter

Table 3 Effect of different dosage regimens on frequency of joint bleeding in patients 5-7

Pat no	Age[a] (y)	Type of F VIII conc	Frequency of infusion	F VIII (units/kg)	Level F VIII (U/ml plasma post inf)	Effect on joint bleeds
5[c]	3 2-3 8	Kabi	1/w	17-28	0 2-0 5	Somewhat reduced frequency
	3 8-4 5	-	-	0	-	Venipuncture difficult 29 treated bleeds
	4 5-5 3	Kabi	1/w	21	-	34 treated bleeds+20 prophylactic treatments
	5 3-6 5	Hematrate	1/w	25	0 4	22 bleeds right elbow and 20 in ankles/feet (esp left)/year
	6 5-7 4	Hematrate	1/w	24	0 35	Somewhat increased freq
	7 4-8	Hematrate	1/w	16	-	Frequent elbow bleeds[d]
	8-9 5	Hematrate	2/w	22	0 45	4 in elbow/year 1½ in ankles/month
	9 5-	Hematrate	3/w	25	0 28	1-2/month
6	*5-7*	Hematrate	1/w	25	0 50	1/month in either foot
	7-7 5	Hematrate	2/w	15	-	Increased bleeding freq
	7 5-8 5	Hematrate	2/w	27	0 44	Only 4/10 months—ankles
7	3 8-5 5	Hematrate	1/w	25	0 48	1-2/month[b]
	5 5-6 5	Hematrate	1/w	20	0 30	None
	6 5-7 5	Hematrate	1/w+1/w	18+9	0 24 (0 12)	4/8 months

[a] Italics = home treatment [b] Additional prophylactic treatment not given when treated for bleeding on previous day [c] Severe psychological problems [d] Gold inj elbow at 7 yrs

(7) the effect with respect to prevention of bleeding was not as good as we had hoped The intervals between treatment episodes were gradually shortened and joint bleeding did not decrease or disappear until levels of F VIII exceeding 0 01 U/ml before infusion and about 0 3 U/ml after infusion were obtained Such a regime has been used elsewhere during recent years (3, 9) Before the home treatment period it was difficult to convince the patients and parents to come to the hospital during all episodes of bleeding and therefore many probably went untreated, or were treated too late to prevent arthropathy Since home treatment was started almost all joint bleeds seem to be treated immediately

It is very interesting to note that among our patients it is the ankle joint which shows the most marked and frequent changes The observed predominance of ankle joint changes compared with changes in the knees described above cannot be attributed to chance The explanation may be that our present treatment with concentrate and physiotherapy prevents functional impairment due to muscle imbalance with atrophy and contractures of especially the knee, which in the past would rapidly leave the patient unable to move Our treatment enables the patients to be up and about and participate in many activities such as skiing, swimming and dancing without major discomfort In turn, this makes them more susceptible to bleeding and changes in the ankle joints In this context we feel that great attention should be paid to the importance of subclinical bleeding as a contributing factor to hemophilic arthropathy since this may be associated with conditions which are unfavorable for metabolism in the joint tissues Ultrastructural studies on synovial membrane in chronic hemophilic arthropathy have shown massive proliferation of smaller blood vessels the walls of which are often defective and predisposed to constant leakage of blood into the joint cavity itself (5) This may explain why progressive destruction occurs in hemophilic joints even though there are no major bleeding episodes

In addition to one patient with a congenital defect of the femur two more patients showed

inequality of leg length which may be ascribed to changes in the epiphyses and the articular cartilage Such problems must not be overlooked

ACKNOWLEDGEMENTS

We thank Drs S Hajder and Bengt E Ginsburg (Depts of Paediatrics Karolinska Hospital and St Goran s Hospital) Dr Helen Walter (Stockholm Blood Centre) and Mrs Lena Rahm for their cooperation

Gold injections into joints were given by Dr Å Ahlberg Malmo

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Submitted Oct 4 1979
Accepted Jan 25 1980

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Acta Pædiatr Scand 69 675-680, 1980

AMPHETAMINE ADDICTION AND PREGNANCY

III One Year Follow up of the Children Psychosocial and Pediatric Aspects

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ABSTRACT Billing, L , Eriksson, M , Larsson, G and Zetterstrom, R (Department of Paediatrics Karolinska Institute, St Goran's Children s Hospital, Stockholm, Sweden) Amphetamine addition and pregnancy Acta Paediatr Scand, 69 675, 1980.—Sixty six infants born to amphetamine addicted mothers were followed during their first year of life The children were divided into three groups according to whether or not the mother stopped her abuse in early pregnancy (Group I) or continued (Group II) and whether or not the latter children were placed in foster homes immediately after birth (Group III) All but 2 of 16 mothers in Group I stayed off drugs and mostly met non addicted friends In Group II, on the contrary, all but 2 of 36 mothers continued their abuse one year after delivery At the age of one year all but one child in Group I were in their mothers' custody and all children in Group III had remained in foster care In Group II one-third of the children lived in foster homes after revocation of the maternal custody Several infants in Group II had experienced multiple transfers between the biological home and different foster homes There were indicators that maternal amphetamine abuse causes temporary drowsiness in the infants during the first months after birth However, all children at the age of 12 months, regardless of group, had a somatic and psychomotor development in accordance with the normal Swedish standard In all groups there was an increased rate of medical care mainly because of infections Some infants in Group II compared to none in Groups I and III were hospitalized because of failure to thrive or suspected physical abuse Symptoms indicating emotional disturbance were more common in infants of Group II than in infants of Groups I and III

KEY WORDS Maternal abuse, amphetamine addiction, pregnancy, neonatal period, infancy

The effects of maternal addiction to opiates on pregnancy delivery and the newborn baby are well documented (16) There are however very few follow up studies of infants of drug addicted mothers and little is known about the long term prognosis of the children In a few studies mainly emanating from USA it has been found that fetal heroin exposure affects growth and behaviour as well as the perceptual and learning processes (12 24) In a Danish study based on 19 infants born to mothers addicted to a variety of drugs the somatic growth as well as gross and fine motor development was found to be normal at 2 years of age (18)

Amphetamine addiction affects the newborn baby (4, 5) To what extent such infants may suffer from long lasting sequelae is poorly elucidated The present study has been undertaken to investigate the somatic and psychological development and social situation of children born to amphetamine addicted mothers during their first year The consequences of fetal amphetamine exposure, parental adjustment and environmental factors have been considered

MATERIAL

The study includes 66 surviving children out of 69 live born to amphetamine addicted mothers during a period of 19 months (two neonatal deaths and one in late infancy) The methods of collecting the patient material has already been reported (11) Most of the mothers had

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This study has been supported by grants from the Swedish Medical Research Council (No 05250) and the Forsta Majblomman Foundation

Table 1. *Classification of 66 infants of amphetamine-addicted mothers, according to fetal exposure and adverse factors*

Adverse factors	Group I	Group II	Group III
Specific drug exposure (*n*)	16	37	13
Early gestation	+	+	+
Entire gestational period	–	+	+
Instability in home environment[a]	–[b]	+[c]	–

[a] Definition parental drug abuse registered by the social agencies
[b] Two mothers relapsed after delivery
[c] Two mothers discontinued after delivery

Table 2. *Family structure and social network in Groups I and II*

	Group I (*n*=16)	Group II (*n*=36)
Relationship of mothers		
Lives alone	3 (19%)	8 (22%)
Lives with a drug free man	11 (68%)***	0
Lives with an addicted man	2 (12%)	28 (78%)
Social network of the families		
Parents	10 (63%)	23 (64%)
Drug free people	13 (81%)***	9 (25%)
Drug addicts	3 (19%)	29 (81%)

*** $p \leq 0.001$

been addicted to amphetamine for more than 5 years when becoming pregnant About 1/3 of them also abused alcohol The maternal background as well as details of pregnancy, delivery and the neonatal period have been described elsewhere (5, 11)

The classification of the children according to fetal drug exposure and social situation during their first year of life is schematically shown in Table 1 In *Group I* the mothers discontinued their amphetamine abuse during the first trimester The newborns remained in their mother s custody In *Group II* the mothers took amphetamine throughout pregnancy The infants were discharged in their mother s custody In *Group III* the maternal addiction continued during the whole pregnancy The infants were discharged to foster homes Some of them were temporarily placed in institutions

Environmental characteristics

All but two mothers in Group II continued their drug abuse after the infant s birth Nineteen have taken amphetamine regularly and 15 have been on drugs for various periods of time Two mothers in Group I who were each living with a drug dependent man have periodically had drug relapses The foster families for the infants in Group III were well established with no known abuse of drugs or alcohol

The family structure and social network of the families in Groups I and II are summarized in Table 2 During the infants' first year, there of the men in Group I and nine in Group II had been or still were in jail following charges concerning drugs The home environments were more unstable and drug saturated in Group II than in Group I

Eight of 16 of the mothers in Group I were employed one year after the infant s birth as compared to only one of 37 in Group II The corresponding figures concerning employed father figures were five in Group I and four in Group II

Follow-up methods

Medical, psychological and social data were obtained from personal interviews with the mother or the foster parent at an age of 12 months Hospital and well baby clinic records, as well as Social Welfare records, were studied and supplemented by interviews with the social worker and child health center nurse The children were examined by the same psychologist in their own environment for a period which varied between 60 and 90 min The psychologist had no information about the background of the child or of the group to which it belonged Gesell s Developmental Schedules were used The evaluation of the emotional development was focused on presence of autism, speech development and wariness to strangers [1]

The psychological development could not be examined in 7 infants (2 in Group I, 3 in Group II and 2 in Group III) One mother in each of the Groups I and II refused to participate The remaining infants lived in foster home more than 500 kilometers from Stockholm In these children some information of the development was obtained from the foster mothers the child health nurses the social workers and in some instances the staff at the day nurseries Data were also collected from the well baby clinic records From the information obtained all 7 infants were considered to have a normal gross and fine motor as well as emotional development

Statistical differences between the three groups were calculated with Fischer s exact test The degrees of significance were tested at the 5%, 1% and 0 1% levels

RESULTS

Somatic health

All infants were followed and given immunizations at the well-baby centers In Groups I and II, the infant's chart, however, often had remarks about missed appointments and difficulties in reaching the family With 2 exceptions, the maternal abuse was known by the nurse

[1] The study has been approved by the Ethical Committee of the Karolinska Institute

Table 3 *Hospital admissions during the first year of life of 66 infants of amphetamine addicted mothers*

	Group I (n=16)	Group II (n=37)	Group III (n=13)
Neonatal period			
Number of admissions	2[a]	12[b]	10
Postnatal period			
Number of infants admitted	3	15	3
Number of admissions (total)	4	24	5
Due to infections	3	13	4
Due to accidents		3	
Due to other causes	1	8	1
Number of hospital stays longer than 7 days		12	1

[a] One infant only kept for observation and returned to the maternity ward
[b] Three infants kept for observation and returned to the maternity ward

Somatic growth was within normal range (± 2 S D) in all but two children at an age of 12 months One child with a normal birth weight and length developed a severe gastrointestinal intolerance and was at one year of age outside −3 S D Of the seven children who were small for gestational age at birth all but one (4 of them in foster homes) showed a catch up growth pattern of 1–2 S D

In 3 children in Group II a malabsorption syndrome was suspected because of loose stools and poor weight gain A special diet was therefore prescribed Two infants in each of the Groups II and III were suspected by the foster parents or the well baby physician of having a hearing impairment When tested by audiologically trained personnel they were all 4 found to be normal Three children in Group II and one in Group III were found to have signs of squint

Information about hospital care during the first year of life is given in Table 3 Many of the infants in Group II were kept in the hospital for prolonged periods because of the poor social situation One of them died at home at the age of 6 weeks with a diagnosis of sudden unexpected death in infancy

Most admissions were due to respiratory tract infections or acute gastroenteritis One infant with severe pneumonia due to RS virus required artificial ventilation One child was admitted because of a vascular ring due to double aortic arch, and another due to epidermolysis bullosa

Out of the 3 injured babies one had a head injury, one a burn requiring hospital treatment for 2 days, and one a fractured femur which required a long treatment period Two of the injuries were considered to be non accidental and were reported to the Social Welfare authorities Two children in Group II were hospitalized for failure to thrive which was considered to be due to the poor psychosocial conditions After discharge to foster homes there was a rapid weight gain

Psychological development

The results of the psychological examination are summarized in Table 4 Fine and gross motor development was normal in the majority of the infants in all three groups In Group II, 10 infants (28%) had manifested emotional disturbances The 2 children in Group I with signs of emotional disturbance were those 2 whose mothers had relapsed into drug abuse In Group III all but one infant showed normal somatic and psychological development

Foster parents of 2 infants in Group II and 5

Table 4 *Psychological development*

	Group I (n=16)	Group II (n=36)	Group III (n=13)
Fine and gross motor development			
Normal	14 (88%)	30 (73%)	12 (92%)
Retarded[a]	2 (12%)	6 (17%)	1 (8%)[d]
Emotional development			
Normal	14 (88%)	26 (72%)	12 (92%)
Symptoms of disturbance[b]	2 (12%)[c]	10 (28%)	–
Hypersensitivity	–	–	1 (8%)[d]

[a] Definition retardation of 2 months or more
[b] Definition presence of autism delayed speech development lack of wariness to strangers
[c] Mothers who had relapsed into drug abuse
[d] One child with cerebral palsy

Table 5. *Measures taken by the Social Welfare Department in the families during the infant's first year of life*

	Group I ($n=16$)	Group II ($n=36$)
Non compulsory measures		
Social welfare	16 (100%)	34 (94%)
Family therapy	1 (6%)	8 (22%)
Lay therapist	1 (6%)	6 (17%)
Placement in day nursery	6 (37%)	8 (22%)
Compulsory measures		
Intensive contact with the family[a]	2 (13%)	15 (42%)*
Child on probation at one year of age	0	8 (33%)
Child in foster home at one year of age	1 (6%)	12 (33%)*

[a] Definition. The social worker met the family 1–2 times every 2 weeks.
* $p \leq 0.05$

in Group III reported that the children had been very slow in their early development and had shown a marked lassitude throughout the first six months of life. As already mentioned, the hearing impairment which was suspected in 4 babies had such an explanation. However, at the age of 6–8 months there was a rapid catch-up in the psychomotor development which was found to be normal at 12 months.

Measures taken by the Social Welfare Department

All mothers were registered at the Social Welfare Office in the district where they lived. The support offered to the families in Groups I and II by the social agencies is presented in Table 5. The families in Group I mainly received support on voluntary basis. The awareness of the more unsatisfactory environment in Group II than in Group I was reflected in the significant differences in number of contacts with the families. Moreover, there were more compulsory interventions in Group II than in Group I according to the Child Welfare Act, i.e. children on probation and revocation of maternal custody. Seven of the 36 infants in Group II experienced four to six temporary periods of separation from their mothers and stayed at institutions or in foster homes for various lengths of time. All the children in Group III were still in foster care when they were one year old. One child had experienced a change of foster parents at 3 months of age.

In addition to the two infants with non accidental injuries, a third case of suspected physical abuse in Group II was reported to the Social Welfare authorities.

DISCUSSION

A normal intrauterine and extrauterine growth has been found following fetal exposure to amphetamine which is in contrast to the findings following fetal exposure to alcohol and opiates (2, 13, 22).

A high rate of hospital admissions during the first year of life was found in the infants in all groups, particularly in infants who were in the care of an addicted mother. The increased demand for health care may be due to maternal insufficiency as well as to known poor social conditions, a well-known cause of frequent hospitalizations and visits to the out patient clinic (8, 10, 20). In accordance to what has been reported in children of heroin addicted mothers (23), infections were the main reasons for admission in all groups.

One infant in the care of an addicted mother died at home at 6 weeks of age with a diagnosis of sudden unexpected death in infancy, a cause of death which occurs at a high rate in infants of heroin-addicted mothers (14, 15).

At 12 months of age the majority of the infants in all three groups had a gross and fine motor development within the normal range which is in agreement with the findings in children born to heroin-addicted women (18, 19, 24), but in contrast to what has been found after fetal exposure to alcohol (2, 13). The symptoms of emotional disturbance, as particularly seen among infants remaining in the care of an addicted mother, may have the same origin as when they occur in a stressful

environment of parental dysfunction of other causes (6, 9) including heroin addiction of the mother (22)

The slow early development or apathy reported to have occurred in some infants born to those mothers who probably had the most severe amphetamine addiction may be a long-lasting effect of the fetal exposure to the drug An institutional deprivation (17) very unlikely was the cause of the transient apathy since all these infants were in foster care before two months of age Nor does it seem likely that the explanation is an over-anxiousness among the foster parents looking for impairments of development

Nevertheless, the occurrence of lassitude in a number of these infants indicates an urgent need for a stimulating and socially beneficial environment for the infants of amphetamine addicted mothers

Despite intensive contact with the addicted families, the social welfare authorities have had difficulties in establishing a confidential relationship with the mothers and in obtaining information of the actual situation The main explanation to this fact may be a fear among the mothers of losing custody of the child Instead reports concerning the children s social conditions very often were given by the police and anonymous sources Most of the families were on public assistance and were provided with proper housing conditions Like other dysfunctional parents in crises (3) the addicted mothers did not always avail themselves of the support offered by the social authorities Housing which was provided may even have had a negative effect when the household became a resort for other addicts

The addicted mother s strong desire to maintain parental right has in this study as in other reports (1 6 12) led to multiple transfers of the children between the biological homes, institutions and different foster homes Considering the need for a psychological parent and continuity in early childhood (7) these recurrent separations must be considered an additional important adverse factor in children who already may be emotionally deprived

In agreement with other studies (1, 21) we have found that drug addicted mothers suffer early disablement in their child caring functions Despite a mobilization of the whole battery of social interventions, the mothers have been unable to provide their infants with a satisfactory environment On the other hand, if the mother is able to give up the abuse during early pregnancy the prognosis for mother and infant seems to be rather satisfactory Emotional disturbances seen in infants of drug addicted mothers at an age of one year mainly seem to be due to unfavourable postnatal psycho social factors The importance of providing newborn infants of drug addicted mothers with the best possible care is obvious In most instances the most rational way must be to place the infants in good foster homes as soon as possible after birth

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One infant in the care of an addicted mother died at home at 6 weeks of age with a diagnosis of sudden unexpected death in infancy, a cause of death which occurs at a high rate in infants of heroin addicted mothers (14, 15)

At 12 months of age the majority of the infants in all three groups had a gross and fine motor development within the normal range which is in agreement with the findings in children born to heroin addicted women (18 19 24) but in contrast to what has been found after fetal exposure to alcohol (2, 13) The symptoms of emotional disturbance, as particularly seen among infants remaining in the care of an addicted mother, may have the same origin as when they occur in a stressful

Acta Pædiatr Scand 69 681–685, 1980

WHAT HAPPENS TO CHILDREN WITH RETARDED SPEECH AT 3?

Longitudinal Study of a Sample of Normal Infants up to 20 Years of Age

GUNNAR KLACKENBERG

From the Clinic for the Study of Children's Development and Health, Department of Paediatrics at the Karolinska Hospital, Stockholm, Sweden

ABSTRACT Klackenberg, G. (Clinic for the Study of Children's Development and Health, Paediatric Department, Karolinska Hospital, Stockholm, Sweden) What happens to children with retarded speech at 3? Acta Paediatr Scand, 69: 681, 1980.—212 children recruited at random at birth have been followed annually in a prospective longitudinal study with somatic and psychological data, including verbal communicative ability, rated by a psychologist at 3 years of age. At the age 20 years 175 were still in the study. Intelligence tests at 5, 8, 11, 14 and 17, teacher's assessments in grades 3 and 6, educational level at 20 years of age and sociometric and behavioural assessments show that children with retarded speech at 3 constitute a risk group that requires special attention.

KEY WORDS: Verbal communicative ability, longitudinal study, mental development

The need to map what happens to children with retarded speech was pointed out some years ago at a multidisciplinary symposium with the Swedish Medical Society arranged on The Incomprehensible Child (9). The work on speech screening in connection with studies of 4-year-olds, e.g. in Uppsala, Goteborg, Varmland and other districts, has yielded simplified methods for identifying children whose speech is inadequate. There are now some 300 logopedists in Sweden and a great deal of their time is devoted to the habilitation of speech retarded children. What has been missing is a long term follow up such children.

The frequent association between appreciably delayed speech development and substantial mental retardation is well known. A consultation for the former is often the context in which the latter is brought to light. Such cases are not however considered here. Instead this study is concerned with the far more numerous children who are burdened by speech delays that are less pronounced.

It is really only prospective longitudinal studies that afford a satisfactory approach to the relation between early speech development and later mental development and school performance. The material for the present report comes from the wealth of data that has been assembled in the longitudinal Solna study. 212 children recruited at random at birth have been followed annually with somatic and psychological data, including speech development (2, 4). At the age of 18–20 years 175 of these children were still in the study. The drop-out is small by international standards as well as in view of the study's special, longitudinal nature. Neither has it significantly altered the socio economic composition of the sample.

METHODS

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Submitted Febr 20 1980
Accepted March 28 1980

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METHODS

The verbal communicative ability at 3 was judged by an experienced psychologist during test situation (a free doll play and Terman Merrill) with the mother in the same room occupied in interview with another psychologist The ratings were *defined* operationally in accordance with a schedule for the internationally coordinated longitudinal studies Rating 1) single words only 2) elementary sentences seldom exceeding 3 words or a few longer utterances interspersed with jargon 3) sentences up to 6

Table 1 *Ratings of speech at 3 years done by a psychologist (percentage distribution)*

Ratings	Girls (n=88)	Boys (n=120)
1	1 1	3 3
2	17 0	29 1
3	50 0	50 0
4	22 7	15 0
5	9 1	2 5

Sex difference 1+2/4+5 p=0 025

or 7 words Still some difficulty in making meaning clear or attempts at verbal expression distinctly limited 4) Simple sentences which are often incomplete but adequate for most practical purposes Or correct constructions but limited vocabulary 5) Mature correctly worded sentences with a vocabulary in advance of the average (The rating procedure is described in ref 5)

Somatic examinations were undertaken by a pediatrician on each occasion

To represent the children s performance the following data were drawn from the large amount of information which has been assembled over the years development of intelligence at 3 8 11 (Terman Merrill) and 14 and 17 years (WIT) teacher ratings in grades 3 and 6 (five point scales) and education completed at 20 (primary and secondary levels) The social data employed included the parents socio economic group (using the Swedish occupational division into three groups as well as Graffar s four groups which allow for income housing and education as well as occupation (3)) The educational level of the mother was used is a separate variable (five point scale primary not completed primary lower secondary upper secondary with matriculation university)

To assess the children s school adjustment use was also made of their sociometric status in the schools they were attending in grades 3 and 5 (10 and 12 years of age) The social matrices that were used to elucidate the children s peer relations are based on replies to structured questions about positive choices of peers for school work and play (1)

Material for the processing of the behavioural variables was obtained from structured annual interviews with the parents up to the child s 5th year and from the corresponding annual questionnaires after that

Statistical methods When comparing groups the t test was applied for the significance of differences between mean IQs The χ^2 test was used for non continuous variables Pearson s method was adopted for assessing correlations Levels of significance are specified in each table

RESULTS

The psychologist s ratings of speech ability at the age of 3 are presented in Table 1 Group 1 has the poorest and group 5 the best speech development Groups 1 and 2 combined constitute the children with retarded speech groups 4 and 5 those with advanced speech It is these two units which feature in the predictive assessments below

When studying these figures it should be borne in mind that this is a sample of somatically normal children None of the speech retarded children had a birth record of cerebral injury or other syndromes associated with inadequate speech development From reliable studies of the incidence of mentally retarded individuals in Sweden one would expect one or, at most, two of the subjects in our sample to manifest a developmental disturbance and a need for psychiatric pedagogic care at some time during childhood and adolescence One child with premature sutural closure and developmental disturbance was in fact detected quite soon, this case does not feature in the statistical analyses of developmental relationships The IQs of the other children were distributed over the entire range Another child was found to have an early neurogenic

caps and went on to matriculate in the social sciences

The social characteristics of the families when the subjects were 3 years of age were as follows for the speech retarded compared with the advanced group

(1) the socio economic status of the parents was lower for the speech retarded group ($p<0$ 02)

(2) the mothers educational level was lower ($p<0$ 02 for girls $p<0$ 05 for boys

(3) all the parents spoke Swedish and the incidence of parents with foreign language as their native tongue was the same in both groups

(4) the proportion of subjects who were the family s only child up to the age of 2 was smaller in the speech retarded group ($p<0$ 01)

Table 2 *Differences in IQ—means through years in same children with advanced, average or delayed speech ratings at 3 years of age*

Statistical probabilities *t* test

Test method	Age (yrs.)	Advanced/delayed ♀	Advanced/delayed ♂	Average/delayed ♀	Average/delayed ♂	Advanced/average ♀	Advanced/average ♂
Terman Merrill	3	0.001		0.001		0.001	
Terman Merrill	8	0.001		0.001		0.001	
Terman Merrill	11	0.001	0.001	0.001	0.001	0.05	n.s.
WIT	14	0.005	0.001	0.01	0.01	n.s.	n.s.
WIT	17	0.02	0.001	n.s.	0.05	n.s.	n.s.

n.s. = no significance

Intelligence tests

For girls as well as boys, significant Pearson correlations of around 0.45 were found between speech development at 3 and the development of intelligence at 8 and 11 years of age. At 14 years, WIT testing gave a significant correlation coefficient of 0.35.

These results apply to the sample as a whole. Table 2 presents the calculated probabilities of differences in mean intelligence at these ages between the three sub-groups for speech development at 3 years of age (delayed, average and advanced).

The delayed-speech group had a poorer initial position and at the age of 17 this group's level of intelligence still lagged behind that of the children whose speech was advanced at an early age. Approximately one half of the girls and one fourth of the boys in the speech-retarded group but only an occasional child in the advanced-speech group had a record of special arrangements at school (i.e. class for immature children, remedial reading or hearing handicap, speech training, or a break from school).

Teacher's assessments in grades 3 and 6

The subjects' school achievement in relation to the rest of the class was rated on a 5-point scale by the teachers in grades 3 and 6 (10 and 13 years of age). In both grades the χ^2 test showed a significant difference ($p<0.001$) between the groups whose speech had been delayed and advanced respectively at the age of 3. The speech-retarded children belonged predominantly to the two poorest ratings for school performance.

Even for the whole sample, a strong relationship was found—as expected—between measured intelligence and school performance as assessed by teachers. Between teacher ratings in grade 3 and IQ at 11 and 14 years, for instance, the correlation coefficients are 0.67 and 0.74 respectively.

Table 3 *Educational level at 20 years (percentage distribution)*

	2 or 3-yr upper secondary school Girls	2 or 3-yr upper secondary school Boys	3-yr upper secondary school Girls + Boys	Started university Girls + Boys
Whole sample	59	71	27	16
Speech-delayed at 3 yrs	31	52	14	5
Speech-advanced at 3 yrs	81	87	37	22

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The social characteristics of the families when the subjects were 3 years of age were as follows for the speech retarded compared with the advanced group

(1) the socio economic status of the parents was lower for the speech retarded group (p<0 02)

(2) the mothers educational level was lower (p<0 02 for girls p<0 05 for boys

(3) all the parents spoke Swedish and the incidence of parents with foreign language as their native tongue was the same in both groups

(4) the proportion of subjects who were the family s only child up to the age of 2 was smaller in the speech retarded group (p<0 01)

of development to above average intelligence at 14 did occur for both sexes (of speech delayed children, 23% had risen above the average by the age of 14 and of the speech advanced 59%)

CONCLUSIONS

In a normal sample the children with delayed speech at 3 years of age constitute a risk group that requires special attention A majority of these children lag behind in school right from the start They are liable to become retarded readers and perform weakly in basic subjects They also tend to deviate more than the aver age in non cognitive respects perhaps to some extent on account of their negative experience Language is a tool for the development of con cept formation and abstractions Normal speech development presupposes both an adequate speaking environment that provides good linguistic models and an emotionally engaged climate that stimulates communica tive interaction and thereby training in the use of language

Linguistic ability and intelligence are closely related and both are affected early by interac tion with parents Many children who start life with inferior intellectual equipment learn to speak later than others and those with a poorer linguistic ability have a weaker and more lethargic substratum for the development of intelligence While it may be difficult to dem onstrate which factor is most important it is clear that a considerable weight should be attached to the environmental side It is there among other things that parents and profes sionals can make a contribution after listening to the speech signals from 3 year olds

ACKNOWLEDGEMENT

The author wishes to express his gratitude to the Radda Barnen Research Foundation for economic support

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Submitted June 18 1979
Accepted March 28 1980

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Educational level at 20 years of age

Data on the educational level achieved at the age of 20 are presented in Table 3 for the 76 girls and 101 boys for whom such information was available. The probabilities of differences in completing upper secondary school (2 or 3-year course) between groups with delayed speech at 3 years are <0.01 for girls and <0.02 for boys The correlation between advanced speech development and upper secondary schooling, expressed as the phi-coefficient, is 0.49 and 0.42 respectively

Adjustment to peers and society

For choice of playmate (from the sociometric test in grade 3), the median for the girls with originally delayed speech differs ($p<0.05$) from that for the girls with advanced speech who are more popular No significant differences in this respect were found in grade 5

For choice of workmate in grade 5 (12 years of age), the children with advanced speech at 3 years of age are preferred to those with delayed speech (median-test, $p<0.05$)

The delayed-speech group was over-represented compared with the advanced-speech group among subjects in the sample who, by the age of 20, had been registered under the Child Welfare Act Such a record generally had to do with minor offences and mostly concerned boys (24 boys, 3 girls) In 6 cases (incl 4 speech-retarded), however, the child had been sent to a reformatory

Behavioural differences at various ages

The variables with distinct symptoms that were chosen to elucidate any behavioural differences at 4, 8 and 12 years of age between speech-retarded and advanced children were enuresis, encopresis, fear of the dark sleep resistance, temper tantrums, destructiveness, stammering and nailbiting A statistical co variation was found for destructiveness at 4 years ($p<0.005$), nailbiting at 8 years ($p<0.05$) and stammering at 12 years ($p<0.05$)

COMMENTS ON THE RESULTS

The chief aim of this study has been to elucidate the subsequent development of the 26% of the children in a normal sample whose speech development was below average at 3 years of age These children have been compared in certain respects with the rest of the sample, primarily with the 27% whose speech was advanced at 3 years Most of those who have studied speech development are agreed that girls are earlier on average than boys (6 7, 8) and this was found to be the case here The two sexes were therefore followed up separately on the whole

For both sexes in this study, in the delayed speech group both the socio economic status of the parents and the educational level of the mothers was lower than in the advanced speech group An earlier paper from this longitudinal study reported a similar finding of a positive relation between speech development at 3 years and the frequency with which parents and children read together at 2 years These observations suggest that differences in early environmental conditions may be reflected in the ability to handle the verbal code

Intelligence tests and teacher ratings clearly show (Tables 2 and 3) that children whose speech is delayed at an early age are not as well equipped as others to meet educational demands As long as speech is still evolving it is hardly surprising that delayed speech should be related to intelligence when this is measured with such a verbally loaded test as the Terman Merrill The unexpected finding was that cognitive difficulties remained so widespread during school years that marked group differences were obtained Right up to the age of 20 delayed speech at 3 carries a clear risk of poorer performance at school Speech development is a signal which can be read at an early age and it may be a good predictor, especially for girls, of subsequent school results Among the boys there were more examples of remarkable improvement from a poor initial position, though instances

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LETTER TO THE EDITOR

Sir

We read with interest (and some dismay) the paper by Solomons and associates—*Acta Paediatr Scand* 78 171 1979 The authors present data to show that excess pulmonary excretion of hydrogen (H) in response to a 10 gram dose of the non absorbable disaccharide lactulose was significantly lowered in children with active gastroenteritis and diarrhoea when compared with non diarrhoeal controls They go on to state (fairly categorically) that the breath H test cannot be recommended for measuring carbohydrate malabsorption in individuals with active ongoing diarrhoea Whilst we agree with their findings—and indeed have found a similar effect in our laboratory we cannot allow their conclusions to go unchallenged for the following reasons

1 On the basis of their own results it should be easily possible to differentiate between carbohydrate malabsorption and normal absorption in the majority of cases The H rise although lower in diarrhoeal patients is still significant when carbohydrate is malabsorbed (the gas chromatography method of H detection should measure down to 5 parts per million (p p m))

2 Three patients in the diarrhoeal group had been on antibiotics which could have been a factor in the reduced H production of the total group Were their H rises the lowest?

3 Statistical analysis of the difference between means where $n=5$ for controls and $n=10$ for the diarrhoeal group may be misleading Unpublished data from our laboratory using larger numbers of children verifies the lowering of the mean but not median H_2 production in the diarrhoeal group but the large standard deviation (S D) renders this difference in mean statistically non significant (See table below) The H_2 response was obtained following ingestion of 6 68 g lactulose in 22 healthy children and 13 children with diarrhoea in whom no antibiotics had been used

	Normal group	Diarrhoea group
Mean H +S D	39 8±44 8 p p m	26 9±24 9 p p m
Range	0–190 p p m	0–98
Median	26	23

We wish to stress that in our experience most patients with ongoing diarrhoea produced a substantial (albeit lowered) rise in breath H when carbohydrate is malabsorbed Possible false negatives are easily confirmed by using lactulose to test hydrogen production The breath H_2 test is therefore *not* rendered inapplicable to the study of carbohydrate malabsorption in diarrhoeal disease—that group where this kind of testing is important

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The Editor has asked Drs Solomons and Viteri to comment on this letter

Sir

We are heartened that our publication (7) has stimulated interest in the evaluation of the H breath test in active diarrheal disease and that Drs Robb and Davidson have substantially confirmed our observations A research team in Bangladesh recently published the results of H breath tests of lactose absorption in 331 rural villagers (4) they also noted a reduction of breath H excretion during active diarrhea (K H Brown personal communication) The phenomenon seems adequately confirmed

It is refreshing to have data from other parts of the world as our results reflect the reality of typical gastroenteritis as seen in Central America Here the patients usually present to

Acta Pædiatr Scand 69 689–690, 1980

SHORT COMMUNICATION

HOST FACTORS AGAINST SALMONELLA TYPHI IN CHILDREN WITH GLUCOSE 6-PHOSPHATE DEHYDROGENASE DEFICIENCY

It is known that glucose-6 phosphate dehydrogenase (G6PD) deficiency can influence the clinical symptoms in some non hemolytic diseases (1 6, 8) It has also been observed that some diseases are more common in subjects with G6PD deficiency (5, 7, 8).

Owusu et al found that in Nigerian patients with typhoid fever the incidence of G6PD deficiency was higher than in the normal population The reason for this predisposition to *S typhi* infection remains uncertain

In this paper we report studies to define some host factors which might be related to the susceptibility of patients with G6PD deficiency in *S typhi* infection

We examined ten boys varying in age from 4 to 10 years In the past they had all suffered from hemolytic crises of favism Ten normal children served as control subjects No subjects had any evidence of infection at the time of study The leukocyte bactericidal activity for *S typhi* and the capacity of serum factors to promote phagocytosis of *S typhi* by normal leukocytes were studied in all subjects as published elsewhere (10) The serum immunoglobulins (IgG, IgA, IgM) and the C_3 and C_4 fractions of *complement* were also estimated Three unselected normal and three mutant subjects were vaccinated with *S typhi* vaccine (vaccine Sclavo, Italy)

The results are shown in Table 1

Comment

Our results show that in the Mediterranean type of G6PD deficiency, there are no abnormalities of host defence against *S typhi* at least in the parameters we studied Our previous studies demonstrated that the leukocyte bactericidal activity against *Staphylococcus aureus* was also normal (10) Although in Sicily there are 1 67% male carriers (11), we have not yet seen any of these with particular susceptibility to infection (10) In white people with G6PD deficiency no relationship between this anomaly and increased infectious diseases or other anomalies have been reported up to now Only in a few cases with absence of G6PD both in the erythrocytes and leukocytes

Table 1 *Results of the immunological studies*

Subjects	Erythrocyte G6PD (U/100 ml)	Leukocyte G6PD (U/mg prot)	Bactericidal activity* Opsonic activity*	Antibody titre*	Fractions of serum complement mg/dl		Serum Immunoglobulins g/l		
					C_3	C_4	IgG	IgM	IgA
Normal	148+8 6	0 38+0 1	90–95	1 100 1 120 1 120	121 7±31	28 7±10	8 8±3 0	1 1±0 2	0 65±0 07
Mutant	1 06±1 8	0 09+0 04	90–95	1 170 1 100 1 100	105 7±30	27 1±10	9 0±1 8	1 0±0 2	0 65±0 1

* Percentage of *S typhi* killed at 60
* Percentage of *S typhi* killed at 60 by normal PMN with opsonins *from mutant* subjects
Four weeks after vaccination

the hospital in advanced states of dehydration after having received a series of home and proprietary remedies, often including antibiotics A more prompt presentation to the hospital of diarrheal cases with lesser alterations of bacterial floral mass, milder intestinal secretion rates, and less self-medication in Australia might possibly better explain the textural differences between the findings for the two regions than sample size, per se.

Clearly, if, as originally described (2), the H_2 breath test is applied in each individual, expressing the response to a test carbohydrate as a percentage of the response to a nonabsorbable carbohydrate, such as lactulose, i e using the patient as his or her own control, application of the H_2 breath test, even in active diarrhea might be valid However, this would require the diarrheal patient to be in a "steady-state" condition during both of the H_2 breath tests? Usually, however, standard diagnostic criteria of a "normal" or "abnormal" H_2 response have been employed The difference in response to lactulose between groups in the data of Drs Robb and Davison confirm our assertion (7) that other than the established diagnostic criteria would be required for evaluation of individuals with gastroenteritis

Most importantly, however, this controversy invokes newer concepts of clinical decision-making theory, it is important to consider the *predictive accuracy* of a test, embodying the concepts of "specificity" and "sensitivity" or the ability of a diagnostic tool to avoid false positive and false-negative diagnoses, respectively (5) Dr Ronald Barr, in a soon to-be-published chapter (1), has applied the concepts of specificity and sensitivity to the use of the H method as a screening procedure for carbohydrate malabsorption at the population level In normal adults, the H_2 breath test has been found to be virtually 100% specific and sensitive for diagnosing lactase deficiency (3 6) Dr Barr correctly points out, however, that any condition that systematically reduces the production of H_2 would decrease the sensitivity of the H_2 breath test, increase the number of false negatives, and lead to an underestimation of the prevalence of carbohydrate malabsorption, if the rate of malabsorption is high (as might be expected in a group of children with gastroenteritis), even small numbers of underproducers in a survey sample would greatly reduce the predictive accuracy of the breath test results and distort the estimation of malabsorbers for that population

Considering all of these factors, we must reiterate our caution regarding the unreliability of the H_2 breath test for carbohydrate malabsorption in a population of individuals with active, ongoing, diarrheal episodes

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Acta Pædiatr Scand 69 691–692, 1980

SHORT COMMUNICATION

PITUITARY-GONADAL FUNCTION IN CHILDREN TREATED FOR ACUTE LYMPHOBLASTIC LEUKAEMIA

Endocrine function, with particular reference to growth, has been studied in children following treatment for acute lymphoblastic leukaemia (ALL) (6) Less data is available on gonadal function, which may be disturbed by the effects of chemotherapy (5) and by prophylactic testicular irradiation (3)

We have studied pituitary-gonadal function in 24 children (12 male) age 8 5 to 19 2 years, of whom 17 had been off treatment for a median period of 3 1 years (range 0 5–4 8) Treatment comprised the MRC therapy trials I-IV (4) which included prophylactic cranial irradiation (range 2000–3000 rads) Basal and peak plasma LH and FSH concentrations following 100 μg bolus I V LHRH were determined by radioimmunoassay Leydig cell function in 11 boys was studied by measurement of plasma testosterone concentrations before and after 3 daily I M injections of 2000 units human chorionic gonadotrophin (HCG)

Results in relation to sex and pubertal stage are shown in Tables 1 and 2 Prepubertal children showed appropriate gonadotrophin responses, although cases 4 and 15, who were still on treatment had an elevated peak LH response An absent LH response in case 5 was also associated with no increase in plasma testosterone after HCG (see later) Elevated peak LH and FSH levels occurred in case 21 (tested in luteal phase of menstrual cycle) and in case 23 (tested 2 weeks after an abortion) Case 9 (peak LH>50 u/L) had received high dose cyclophosphamide therapy

Prepubertal boys showed normal plasma testosterone increments after HCG stimulation (Table 2) A subnormal response in case 5 was anticipated because of previous orchidectomy and contra-lateral testicular irradiation, but there was an unexpected absent LH response to LHRH (Table 1) Case 9, who had received high dose cyclophosphamide therapy, showed no testosterone response to HCG in keeping with his elevated peak LH response. The 3 postpubertal boys showed normal Leydig cell responsiveness, but all demonstrated oligo- or azoospermia on semen analysis

Most prepubertal children in this study showed normal pituitary-gonadal function A

Table 1 *Basal and peak serum LH and FSH levels after LHRH stimulation*

Case no	Puberty stage	LH (U/L)		FSH (U/L)	
		Basal	Peak	Basal	Peak
Males					
1	P1	<0 7	4 8	1 0	2 6
2	P1	<0 7	3 0	1 8	7 2
3	P1	<0 7	5 9	<0 3	2 0
4	P2	<0 7	13 8	2 5	8 2
5	P2	<0 7	<0 7	<0 3	2 1
6	P2	<0 7	18 2	0 9	4 3
7	P3	<0 7	33 4	6 4	17 2
8	P3	1 6	21 4	1 2	4 3
9	P3	0 8	>50	1 7	4 9
10	P5	1 6	24 5	2 5	8 1
11	P5	3 6	22 4	0 5	2 6
12	P5	4 5	24 8	5 0	10 6
Females					
13	P1	<0 7	2 9	<0 3	3 2
14	P1	0 8	3 5	1 5	5 5
15	P1	<0 7	23 0	2 8	19 2
16	P1	1 4	1 5	0 9	1 6
17	P2	1 6	26 8	1 3	9 4
18	P2	2 4	25 8	3 0	10 2
19	P3	0 8	40 4	<0 3	6 6
20	P4	1 6	11 2	0 6	16 5
21	P5	7 9	>50	1 9	24 4
22	P5	5 7	12 7	2 9	4 9
23	P5	23 6	>50	13 5	>40
24	P5	1 3	14 5	<0 3	2 8

In most cases peak serum gonadotrophin levels occurred 30 min after LHRH injection

was there decreased leukocyte bactericidal and metabolic activity with a clinical picture of chronic granulomatous disease (3, 4) However, these cases could be due to a different, severe enzymatic variant, possibly deletion or a defect in transcription On the contrary, in Nigerian children an increased incidence of Salmonellosis (8), viral hepatitis (7) and nephrotic syndrome (5) was seen Petrakis et al (9) found a significant decline of incidence in G6PD deficiency among old black people in the San Francisco Bay area, suggesting that "G6PD deficiency appears to define a group at higher risk of mortality than the general population" However, a recent large survey (4) among black veterans admitted to hospitals failed to demonstrate increased incidence of disease or higher fatality rate in G6PD deficient subjects

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Acta Pædiatr Scand 69 691–692, 1980

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Table 1 *Basal and peak serum LH and FSH levels after LHRH stimulation*

Case no	Puberty stage	LH (U/L) Basal	LH (U/L) Peak	FSH (U/L) Basal	FSH (U/L) Peak
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1	P1	<0 7	4 8	1 0	2 6
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4	P2	<0 7	13 8	2 5	8 2
5	P2	<0 7	<0 7	<0 3	2 1
6	P2	<0 7	18 2	0 9	4 3
7	P3	<0 7	33 4	6 4	17 2
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9	P3	0 8	>50	1 7	4 9
10	P5	1 6	24 5	2 5	8 1
11	P5	3 6	22 4	0 5	2 6
12	P5	4 5	24 8	5 0	10 6
Females					
13	P1	<0 7	2 9	<0 3	3 2
14	P1	0 8	3 5	1 5	5 5
15	P1	<0 7	23 0	2 8	19 2
16	P1	1 4	1 5	0 9	1 6
17	P2	1 6	26 8	1 3	9 4
18	P2	2 4	25 8	3 0	10 2
19	P3	0 8	40 4	<0 3	6 6
20	P4	1 6	11 2	0 6	16 5
21	P5	7 9	>50	1 9	24 4
22	P5	5 7	12 7	2 9	4 9
23	P5	23 6	>50	13 5	>40
24	P5	1 3	14 5	<0 3	2 8

In most cases peak serum gonadotrophin levels occurred 30 min after LHRH injection

Table 2 *Plasma testosterone levels before and after HCG stimulation*
– = not tested

Case no	Puberty stage	Plasma testosterone (nmol/l) Before	After
1	P1	0 4	6 0
2	P1	0 5	6 0
3	P1	0 8	5 0
4	P2	1 7	7 0
5	P2	0 7	4 0
6	P2	–	–
7	P3	3 5	29 0
8	P3	4 5	22 0
9	P3	5 0	5 0
10	P5	19 0	40 0
11	P5	27 0	34 0
12	P5	23 0	38 0

few pubertal boys were abnormal, possibly the result of testicular and cranial irradiation (case 5) and chemotherapy-induced gonadal dysfunction in case 9 The post pubertal boys appeared to demonstrate normal Leydig cell function, but evidence of germ cell damage However, further studies are required later, since recovery of spermatogenesis can occur with time (1) Male fertility after successful chemotherapy for ALL has recently been reported (2) All 4 post-menarchal girls had regular menses and one had proven her fertility by needing a termination of pregnancy for social reasons The results of this study indicate an optimistic prognosis for fertility in girls treated for ALL, but in males the outlook will depend on the capacity for recovery from any previous germ cell damage

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Acta Pædiatr Scand 69 693–694 1980

SHORT COMMUNICATION

EARLY DIAGNOSIS OF OCCULT MEDULLARY CARCINOMA OF THE THYROID BY IMMUNOREACTIVE THYROCALCITONIN DETECTION

Medullary carcinoma of the thyroid (MCT) constitutes 5% to 10% of all thyroid cancers (1) Morphological and histochemical evidence shows that the tumor is derived from nests of parafollicular cells or C cells which are of neural crest origin (8) Both normal and neo plastic C-cells secrete the calcium regulating polypeptide calcitonin and in patients with extensive disease high basal serum concentra tion of thyrocalcitonin can be found (9) MCT may also produce other hormonal substances such as ACTH serotonin carcino embryonic antigen (CEA) catecholamines and prosta glandins (6 7) The association of MCT pheocromocytoma and mucosal neuromas has been designated multiple endocrine neoplasia (MEN) type 2B (1) MCT is frequently a familial disorder inherited as an autosomal dominant trait (1 10) A case is reported of a 12 year old girl who was admitted to our Department in 1977 with a diagnosis of con dylomatosis Her mother had bilateral club-feet and knock knees Her 1 year old brother also had bilateral club feet At four months of age the patient had been diagnosed to have bilateral dysplasia of the hip with dislocation At 5 years she had a gingival nodule removed without histological examination At 7 years of age several nodules appeared over the lips tongue palate and rims of eyelids Since then she complained of chronic constipation and recurrent dysphonia On admission the pa tient measured 139 cm (50th percentile) and weighed 31 kg (50th percentile) The body habitus was Marfan like with dorsal scoliosis dorsal kyphosis and long thin limbs Bilateral club feet and knock knees were observed as well as a peculiar facies with patulous lips prominent jaw and thickened eyelids Multiple mucosal neuromas varying in size from that of a pinhead to several millimeters in diameter were evident over the lips tongue and edges of the eyelids No palpable masses were present on the neck All routine laboratory examinations revealed normal values A barium enema with air contrast showed a large dilated colon likely the result of diffuse ganglioneuromatosis of the bowel Biopsy of a tongue nodule showed plexiform neuromas The patient was discharged with a diagnosis of multiple mucosal neuromas and followed as an outpatient periodically with thyroid scans and determinations for immunoreactive thyro-calcitonin serotonin ACTH PTH CEA vanilmandelic acid (VMA) catecholamines 5 hydroxyindol acetic acid (5 HIAA) All thyroid scans (Tc 99) revealed normal contour and configuration of both lobes Twenty four hour urinary excretion of VMA catechol amines 5 HIAA and serotonin were in the normal range at each control as were serum levels of ACTH and PTH The plasma con centration of immunoreactive thyrocalcitonin was measured by radioimmunoassay (Kit Set Immuno Nuclear Corporation) on peripheral blood samples (2 3) during basal conditions and following two provocative tests with pentagastrin and alcohol stimulation (4 11 12) Normal thyrocalcitonin values with this assay are 25–100 pg/ml Immunoreactive thyrocalcitonin levels were first noted to be elevated 12 months after her discharge (basal

value 7500 pg/ml, after pentagastrin injection 12000 pg/ml, after alcohol ingestion 10000 pg/ml) Serum CEA concentration of 32 ng/ml was also above normal (n v 1–15 ng/ml) At the age of 14 years the patient underwent total thyroidectomy and the histopathologic examination confirmed the diagnosis of bilateral MCT

Comments Medullary carcinoma of the thyroid is regarded as an intermediate grade malignancy between anaplastic and follicular carcinoma (1) The survival time depends on the occurrence of metastases at the time of first surgery, with a 10 year survival of 46% for patients with cervical metastases as compared to 86% for those without metastases (1) Since radiotherapy and chemotherapy appear to be ineffective (5), surgery is the only mode of therapy However early detection of the tumor, in patients at risk for MCT, is possible by monitoring the serum level of thyrocalcitonin, which is abnormally released by malignant C cells (11) In our patient with the marfanoid mucosal neuroma phenotype high serum concentrations of thyrocalcitonin showed to be diagnostic in presence of normal routine analysis and thyroid scans

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Acta Pædiatr Scand 69 695–698, 1980

CASE REPORT

ULTRASONOGRAPHY IN THE DIAGNOSIS OF NEONATAL ADRENAL HAEMORRHAGE

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ABSTRACT Nordshus, T and Monn, E (Department of Radiology, Section of Paediatric Radiology and Department of Paediatrics, Rikshospitalet, The National Hospital, University of Oslo, Oslo, Norway) Ultrasonography in the diagnosis of neonatal adrenal haemorrhage Acta Paediatr Scand 69 695, 1980.—Two cases of neonates with adrenal haemorrhage are presented The clinical, radiological and ultrasonographical signs are described The future role of echography as compared to other radiological procedures, including computerized tomography, is discussed Ultrasonography is recommended as a screening method, and is well suited for assessing the size and internal structure of pathological processes

KEY WORDS Newborns, adrenal haemorrhage, diagnosis, radiology, ultrasonography

In a large autopsy series (2) adrenal haemorrhage is reported to occur in 1 7 of 1000 births The symptoms may be vague and uncharacteristic and the number of children surviving is unknown The figure given thus represents an underestimate and suprarenal haemorrhage or haematoma is probably a relatively common disorder of the neonate (1)

There have been a few recent reports of the ultrasonic diagnosis of this disease (4–8) but to our knowledge no such reports have been presented in Scandinavian literature

CASE REPORTS

Case 1

K O female was born September 1978 after a normal pregnancy length 57 cm weight 4930 g The delivery of the shoulders was difficult and the right humerus fractured She was intubated and ventilated for 10 min before spontaneous ventilation started During feeding on

spleen were palpable just below the costal arches but no abdominal tumor could be felt Haemoglobin was 180 g/l and serum electrolytes were normal There was a microscopic haematuria and a moderate pyuria but no bacterial growth in the urine (On day 14 the urin was normal)

with a translucent central area surrounded by a rim of more dense tissue (Fig 1).

The same day an ultrasound examination of the abdomen revealed a mass in the right upper part of the abdomen between the right liver lobe and the right kidney (Fig 2) with well delineated borders towards both organs The mass consisted of an echofree ovoid central area surrounded by a narrow zone with more echoes In addition a small suprarenal mass was observed on the left side (Fig 3).

The clinical condition gradually improved and the child was discharged 25 days old She was psychomotorically normal with normal growth at a follow-up examination at 6 months of age The clinical examination as well as the IVP was negative

Case 2

H M female was born April 1979 after a normal pregnancy and delivery length 51 cm weight 4590 g She was slightly cyanotic during feeding and was examined by a paediatrician when 2 days old He noticed an enlarged liver (2 cm below the costal arch), and a somewhat enlarged kidney that appeared to be displaced downward with its lower pole at the height of the umbilicus She was jittery and a serum calcium level of 1 5 mmol/l was observed with good clinical response to intravenous calcium Haemoglobin was 180 g/l serum

value 7500 pg/ml, after pentagastrin injection 12000 pg/ml, after alcohol ingestion 10000 pg/ml) Serum CEA concentration of 32 ng/ml was also above normal (n v 1–15 ng/ml) At the age of 14 years the patient underwent total thyroidectomy and the histopathologic examination confirmed the diagnosis of bilateral MCT

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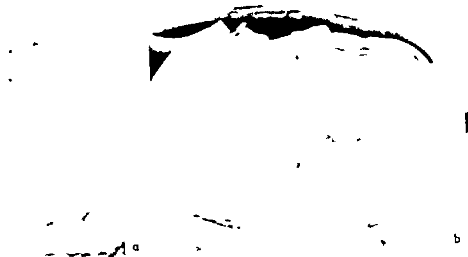


Fig 4 a Ultrasound examination (case 2), longitudinal section through the right kidney (*K*) right liver lobe (*L*), right hem diaphragm (*D*) and large mainly echofree suprarenal expansion (arrowheads). b Ultrasound examination (case 2), transversal section through the left kidney (*K*) liver (*L*) spine (*C*), and large mainly echofree process (arrowhead) paravertebrally on the right side

ditions may be secondary to neonatal asphyxia Adrenal haemorrhage however is often seen in a large newborn after traumatic delivery

Intravenous pyelography has been the classical method for demonstrating a suprarenal mass with a relatively translucent area during the total body opacification phase and a downward displacement of the kidney A similar picture may be obtained with supra renal abscesses and necrotic tumors (4) but these conditions are very rare in newborns

IVP is an invasive method with some side effects and more importantly smaller haematomas may be missed as in case 1 The method is of little help in shocked oliguric patients or in patients with much bowel gas hiding the opacification Aortography adds little information to IVP and has no place in the diagnostic work up (3)

An ultrasonographic technique has been described (7–9) whereby even normal adrenals are demonstrated in more than 90% of the cases also in neonates The echographic examination usually does not even demand premedication and can be easily performed without inconvenience or hazard to the patient Thus it is an almost ideal screening and control method in suspected adrenal haemorrhage

In both our cases, one with, and the other without palpable tumor, and both with vague symptoms the ultrasonography confirmed the diagnosis done by IVP Moreover in case 1, ultrasonography demonstrated a small left-sided adrenal mass, that was missed by IVP



Fig 5 Computerized tomogram 14 days after onset of the symptoms (case 2) through round expansion (arrow) in the right part of the abdomen with attenuation values 10–12 H U (Hounsfield Units).



Fig 1 Intravenous pyelography (case 1) total body opacification phase A suprarenal mass (arrowheads) with a more translucent central area is dislocating the right kidney (*) caudally

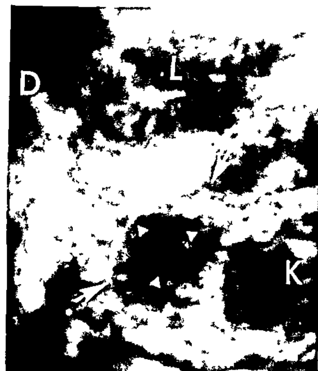


Fig 3 Ultrasound examination (case 1), longitudinal section through the left kidney (K), left hemidiaphragm (*D*), left liver lobe (*L*) and small suprarenal mass (arrows) with relatively echofree central area (arrowheads)

electrolytes were normal, and she developed a transitory jaundice with peak bilirubin at 306 mmol/l on the fifth day of life She had a microscopic haematuria She was in good clinical condition, but was referred to our department for investigation when 4 days old

The clinical examination revealed findings as on day 2, and an IVP showed an expansion above the right kidney with a relatively dense rim surrounding a less dense centre, analogous to the findings in case 1



Fig 2 Ultrasound examination (case 1), longitudinal section through the right kidney (large arrowhead), right hemidiaphragm (*D*), right liver lobe (*L*) and suprarenal mass (big arrowheads) with sonolucent central area (small arrowheads)

Ultrasonography on day 12 showed a mainly echo free expansive process between the right upper renal pole and the right liver lobe (Fig 4*a* and *b*)

Computerized tomography of the abdomen on day 14 revealed below the right liver lobe a round expansion with low attenuation values (approximately 10 Hounsfield Units) (Fig 5)

She was discharged 20 days old At 6 months of age, she was normally sized and psychomotorically normal The clinical examination was negative A plain film of the abdomen revealed a 2×2 cm round slightly calcified expansion above the right kidney which was no longer displaced

DISCUSSION

The symptoms of neonatal adrenal haemorrhage may be fulminating and fatal in bilateral cases, but most often the disease has milder symptoms, particularly in unilateral cases The clinical symptoms may mimic septicaemia, but most of the patients have a palpable abdominal mass (3, 4, 5) As many of the patients with adrenal haemorrhage also show microscopic haematuria, renal vein thrombosis is an important differential diagnosis. Both con-



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Thus, case 1 had a bilateral haematoma with the potential danger of developing Addison's disease months or years later, and she must be controlled accordingly Although it is generally stated that the later appearance of a calcified suprarenal mass confirms the diagnosis of adrenal haemorrhage our case 1 did not show calcification after 6 months

In our case 2, computerized tomography verified, by the attenuation values observed, that the cystic content could represent a 14 days old haematoma Heavy sedation or anesthesia is usually necessary to avoid motion artefacts during the CT examination, especially when using CT scanners with a scanning time of more than 3–4 sec One would expect that CT could give a more specific early diagnosis verifying that a mass contains mainly blood

In our opinion ultrasonography ought to be first examination when an adrenal haemorrhage is suspected, i e before an IVP which in some cases may be avoided Ultrasonography will also play a most important part in the early follow up of these patients Computerized tomography is indicated when the existence of a mass has been verified and when there is sufficient doubt as to the etiology

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Submitted Febr 4 1980
Accepted March 18 1980

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Acta Pædiatr Scand 69 699–703, 1980

CASE REPORT

NEUTROPHIL CHEMOTACTIC DEFECT AND HYPOGAMMAGLOBULINEMIA

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ABSTRACT Lorente, F , Fontan, G , Garcia Rodriguez, M C , Alba J and Ojeda, J A ([illegible] Department of Paediatrics and Department of Pathology, La Paz [illegible] covery, the patient s immunological study disclosed a persistent neutrophil chemotactic [illegible] and hypogammaglobulinemia Other studies of specific and non-specific immunity were normal Neutrophil adherence random and random stimulated mobility were always within the normal range The presence of chemotactic inhibitors was discarded *In vitro* incubation of his neutrophils with Cytochalasin B at 0 1 μg/ml final concentration, reversed the chemotactic abnormality suggesting a possible cell membrane defect

KEY WORDS Chemotaxis hypogammaglobulinemia, immunodeficiency diseases

Persistent abnormal leucotactic movement has been observed in several patients affected by primary immunodeficiencies (4, 9, 16, 23) The incidence of this association remains unknown since the routine immunological study in most of the immunodeficient patients does not include leucotactic studies

Recently we had the opportunity to study a boy with low serum immunoglobulin (Igs) levels and defective neutrophil (PMN) chemotactic response

CASE HISTORY

This boy now 4 years old was the third son of healthy parents who are first cousins He had been in good health until 13 months of age when after an acute spasmodic laryngitis was treated with Chloramphenicol and *Amynopirine* developing fever and a widespread pruritic erythematous rash The patient was noted to have a leucocyte count of 1 8×10⁹/l with 89% lymphocytes 7% PMNs and 4% monocytes Platelet and red cell counts were normal Six days later the PMN count began to increase reaching normal values after one week At this time blood chemistries routine urine analysis and a bone marrow smear did not show any abnormality No antineutrophil antibodies could be found in his serum The immunological stud es (discussed below) disclosed an hypogammaglobulinemia and a defective PMN chemotaxis Following discharge from the hospital he has suffered from minor complaints and a mild infectious hepatitis

An inguinal lymph node biopsy performed at 45 months of age showed a lymphocyte depletion in the cortical region Rudimentary follicles devoid of germinal centers were seen in the cortex No plasma cells showing fluorescence with labelled polyvalent antiserum to Igs could be detected

MATERIAL AND METHODS

The studies were carried out while both the patient and the controls were free of clinical infections and no drugs had been administered during the previous weeks A pool of normal serum was obtained from 14 healthy laboratory personnel

Specific immunity studies Immunoglobulins G A M D and complement components C_4 C_3 and C_5 were determined by radial immunodiffusion IgE was determined by direct sandwich radioimmunoassay Serum hemolytic activity (15) T cell rosettes active (26) and total (19) lymphocyte phytohemagglutinin reactivity (12) B cells with complement receptors (22) and bearing surface stable Igs (13) were determined as previously described

PMN studies In vitro chemotaxis was estimated by a modification of Boyden s technique (8) using plastic disposable microchambers (Mark II Engineering Co Chicago Ill) and Millipore filters 3 μm pore size diameter The chambers were incubated for 3 hours in a humidified atmosphere containing 5% CO_2 As cytotaxins

Table 1. *Serum immunoglobulin levels*
ND = not done

	Age (months)						
Igs (mg/100 ml)	15	18	24	28	31	33	45
G	304[a]	320[a]	500	300[a]	400[a]	325[a]	470
A	6[a]	12[a]	15[a]	20[a]	17[a]	34	13[a]
M	12[a]	38	60	120	94	34	36
D	ND	ND	4 7	4 7	5 6	ND	6 2
E	ND	ND	ND	ND	ND	ND	5[b]

[a] Abnormal values for that age [b] I U /ml

denaturated casein (5 mg/ml) and zymosan activated serum (25), were used The chemotactic index (CI) was evaluated counting the number of PMN accumulated in 16 randomly selected high power fields in the lower side of the filter In some experiments, the distance in μm that the two leading cells had migrated into the filter was determined with the microscope micrometer (28) Chemotactic studies were also performed employing filters of larger pore size (5 and 8 μm) The incubation time giving optimal results for each pore size was previously determined in healthy volunteers Random movement was assessed by eliminating the chemotactic stimulus and using filters of 8 μm pore size Stimulated random migration was measured by placing a similar cytotaxin concentration in both compartments of the chamber The possibility of cell directed chemotactic inhibitors was evaluated by comparing the response of control PMN previously incubated (30 min at 37°C) in either the pooled serum or the patient s serum The presence of serum cytotaxin inactivators was tested by incubating (30 min at 30°C) the cytotaxin with either the patient s or the pool serum The possible deficiency in a serum cofactor necessary for a normal chemotaxis was evaluated incubating (previously to the chemotactic assay) patient s PMN in pooled serum (fresh or inactivated) The *in vitro* effect of Cytochalasin B (Sigma Chemical Co St Louis Missouri) and Levamisole (Jenssen Beerse Belgium) on PMN chemotaxis was examined by preincubating (30 min at 37°C) the control s or patient s PMN in Medium 199 containing different drug concentrations

Neutrophil adherence to glass beads (14) nitroblue tetrazolium dye reduction (8) cellular and humoral components of phagocytosis and intracellular killing to *Staph aureus* and *C albicans* were studied by previously described methods (11 21) All cytochemical stains were performed on peripheral blood The following stains were used Peroxidase alkaline phosphatase periodic acid schiff (PAS) β glucoronidase and naphtol ASD acetate esterase (20)

RESULTS

Serum Igs levels were subnormal, or in the low normal range, during the 30 months follow up (Table 1) Isohemagglutinin and heterophil agglutinin titers were absent or very low, as well as those antibodies against common pathogens such as *E coli* and *C albicans* A measurable antibody response to tetanus toxoid could not be demonstrated in spite of repeated immunization The percentage and absolute number of T and B lymphocytes and the phytohemagglutinin stimulatory index were always within normal range Positive skin tests were elicited with Streptokinase and Mantoux antigens

Table 2 resumes the results of PMN mobility studies performed during the follow up Chemotactic indexes evaluated by counting the cells in the lower side of the filter or by the "leading front" method, were persistently abnormal Random and random stimulated mobilities yielded normal results The use of larger pore size filters did not modify the CI The drug cytochalasin B at 0 1 μg/ml enhanced the chemotactic response especially on the patient s cells which reached a normal CI Levamisole at 1×10^{-4} M concentration did not show any effect on the patient s chemotactic response (Table 3)

Complement studies nitroblue tetrazolium dye reduction serum opsonic activity PMN phagocytosis and bactericidal capacities against *Staph aureus* and *C albicans* were normal in all instances

DISCUSSION

Only a few patients with hypogammaglobulinemia have been found to have intrinsic cellular defect of PMN chemotaxis (5, 23)

Table 2 *Evaluation of neutrophil mobility*

	Patient	Day s control
Chemotaxis		
Cytotaxins		
Casein (187 5±39 3)[a]	26	171
Activated pool serum (168 9+24 3)[a]	41	204
Leading front at 50 min (μm) (87 2±9 5)[a]	56 5	90 1
Different pore sizes		
Size incubation time		
3 μm 3 h	26	171
5 μm 2 1/2 h	38	175
8 μm 2 h	39	176
Random motility (8 μm pore size) (143 6±39 1)[a]	120	145
Random stimulated (3 μm pore size) (77 2±29 3)[a]	37	82 5
Adherence (80 48±8 35)[a]	74 4	72 2

[a] Normal values in our laboratory (Mean±S D)

Transitory hypogammaglobulinemia was the most likely diagnosis when the boy was studied for the first time, but lately, as in the common variable form of immunodeficiency, his Igs levels showed to be fluctuating rather than increasing with age Moreover, his abnormal antibody response and the depleted B cell area showed in the lymph node biopsy, militates against a transitory defect (24)

The assay of PMN movement disclosed a cell intrinsic chemotactic defect with normal random mobility At least three prerequisites are necessary for a normal directed migration (6) adherence of the cell to the substratum, random migration and cell deformability The first two conditions were unimpaired but we have not directly measured the PMN deformability The finding of normal random and random stimulated mobility, and the fact that the impaired chemotaxis was not corrected *in vitro* when larger pore size filters were used, makes unlikely a cell deformability defect (17) Our patient does not seem to have a severe alteration in the microtubule system, as a normal microtubule assembly is necessary for a correct adherence (3), and bactericidal activity (7) Nor does he fit into the category of the actin dysfunction reported by Boxer et al (2) We can not exclude alterations in the membrane receptors for the chemotactic signals, in the subsequent transducer mechanisms, or in the complex metabolic events necessary for a normal chemotactic response The drug Levamisole that enhances the cell movement sustaining high cGMP levels (1) had no effect on our patient's chemotactic response Another drug, Cytochalasin B at high concentrations has an inhibitory effect on cell movement, impairing its microfilament function At lower concentrations, between 0 1–1 μg/ml, the drug has an enhancing effect on chemotaxis These concentrations do not affect the microfilament structure, and its main action is on membrane transport (27) Albeit speculative it is extremely provocative to suggest that the *in vitro* cor-

Table 3 *Drug studies on chemotaxis*

	Chemotactic indexes	
	Patient	Day s control
Basal	32	140
Cytochalasine B		
5 μg/ml	0	0
0 1 μg/ml	140	166
0 01 μg/ml	88	160
Levamisole 1×10 [illegible] M	48	180

rection of our patient s chemotactic abnormality by a low concentration of this drug, could be by correcting a defective cell membrane permeability Recently, a child with severe combined immunodeficiency had his lympho cyte unresponsiveness to mitogens corrected *in vitro* by the addition of the Ionophore A 23187 A cell membrane alteration was pro posed as the most likely explanation (10)

We do not know if our patient s complex defect, affecting two cell lines is primary or secondary to the acute neutropenia but we have not found any other report on hypo gammaglobulinemia or chemotactic abnormalities following acquired neutropenias Our studies do not define a single pathogenic defect acting on both cell lines, but they emphasize the need for detailed PMN function studies on immunodeficient patients

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Submitted Nov 26 1979
Accepted Jan 31, 1980

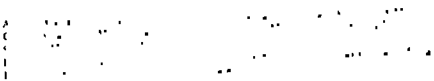
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CASE REPORT

CONGENITAL NEPHROTIC SYNDROME DETECTED BY HYPOTHYROID SCREENING

J T FINNEGAN, E. J SLOSBERG D C POSTELLON and W A PRIMACK

Fr
Way [illegible]



thyroid binding globulin Congenital nephrosis can be [illegible] grams which use thyroxine as the primary screening test, but not by those which use TSH. This may be important in those countries in *which the incidence of* congenital nephrosis approaches that of congenital hypothyroidism

KEY WORDS. Thyroid screening, hypothyroidism, congenital nephrosis, thyroxine, thyroid *stimulating hormone*, thyroid binding globulin

Congenital hypothyroidism is detected reliably by screening programs using either thyroxine (T_4) or thyroid stimulating hormone (TSH) as the primary screening test (4) Recently we evaluated an infant with low T_4 and physical findings consistent with hypothyroidism. She was found to have the congenital nephrotic syndrome, and to be euthyroid with low levels of thyroid binding globulin (TBG), presumably due to urinary losses of this protein (1, 5)

CASE REPORT

A 3019 g female was born at term to a 22 year old primigravida after an uncomplicated pregnancy The mother is of Puerto Rican and the father of Dutch ancestry The neonatal period was uneventful and she was maintained on breast feeding The routine filter paper T_4 screen performed by the Michigan Department of Public Health was 16 7 nmol/l (normal=70 8–148 0) with a TSH of 8 4 10^{-3} int units/l (normal=0–9) These results were confirmed on venous blood Free T_4 was found to be 0 019 nmol/l (normal 0 010 to 0 033) and TBG 38 6 nmol/l (normal 190–320) A radionucleide thyroid scan was normal Urinalysis showed 3+ protein and 50 RBC/high power field with 24 hour urine protein excretion of 1 2 g Creatinine clearance was 0 9 ml/sec/1 73 m^2 with a serum creatinine of 26 5 micromol/l and blood urea nitrogen of 1 1 mmol/l Serum albumin was 116 micromol/l (8 g/l), total protein 28 g/l, and cholesterols 6 2 mmol/l Intravenous urogram was normal Heavy metal screen and serologic test for syphilis were negative Antibody titers to toxoplasmosis herpes, rubella and cytomegalovirus were all negative (less than 1 8)

Because of the urinary abnormalities, a renal biopsy was obtained at 3½ months of age Physical examination at that time showed a healthy appearing infant with height in the 15th percentile weight 10th percentile, and head circumference 40th percentile She had large open anterior and posterior fontanelles with wide sutures a depressed nasal bridge, thick and coarse hair, dry skin soft protuberant abdomen with ascites, a large umbilical hernia, and pitting edema of both lower extremities (Fig 1) Bone age was 3 months The renal biopsy showed moderate mesangial proliferation with areas in the superficial cortex of distended proximal tubules filled with proteinaceous material Immunofluorescence was negative for IgA, IgM and C_3 These findings were interpreted to be consistent with early changes of the congenital nephrotic syndrome (6)

DISCUSSION

A striking similarity exists between the physical and initial laboratory findings of this infant with congenital nephrotic syndrome and those expected in infants with hypothyroidism These include wide sutures with a large patent posterior fontanelle, coarse hair, depressed nasal bridge, dry skin, abdominal distention with an umbilical hernia, and edema (6, 8) In addition, although not seen in this case, respiratory distress, poor feeding, and decreased activity have been reported as features of both syndromes (8) She had a low T_4 and high normal TSH suggestive of hypothyroidism, although the TSH is usually much higher in congenital hypothyroidism (4, 7) These findings, combined with evidence of euthyroid function as indicated by the availability of thyroxine, the free T_4, suggested TBG deficiency, (3, 4) which was subsequently confirmed However, congenital TBG deficiency does not explain the edema Consequent examination of the urine revealed marked proteinuria and microscopic hematuria with the resultant hypoalbuminemia and hypercholesterolemia characteristic of nephrosis Renal biopsy was compatible with the diagnosis of congenital nephrotic syndrome Urinary losses of TBG and thyroxine (1 5) probably led to the low serum TBG and low T_4

The thyroid screening program in Michigan routinely reports newborns with low T_4 levels to the attending physician along with a TSH follow up If TSH were used as the primary screen for hypothyroidism, this patient would have gone undetected until her nephrosis became more troublesome

In summary, we have observed an unusual presentation of congenital nephrosis and we wish to alert physicians to the striking clinical and laboratory similarities between this and hypothyroidism, and stress the importance of ruling out proteinuria and measuring serum TBG whenever any question arises The importance of considering this diagnosis is especially important in areas of the world such as Finland, where the estimated incidence of the congenital nephrotic syndrome approaches one-third that of congenital hypothyroidism and is only slightly less frequent than the occurrence of congenital TBG deficiency (2, 3, 4, 6)

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Submitted Febr 18 1980
Accepted April 3 1980

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BOOK REVIEWS

F H Stone & C Koupernik *Child psychiatry for students* 2nd ed Churchill Livingstone London 1978 £2 25 ISBN 44301797 2

The experienced clinicians, Stone and Koupernik present the second edition of their textbook in child psychiatry, Child Psychiatry for Students, just four years after the first edition appeared Developments during the last few years especially in research on biological factors and psychopharmaca justify this

The book is intended primarily for use in the education of medical students and is meant as an introduction to the topic of child psychiatry The book therefore makes no claims to cover thoroughly the subject matter in this area but rather to present the principles To cover all of child psychiatry in slightly more than 100 pages might appear to be a risky venture Chances are great for impracticable simplifications and sweeping generalizations Much to the authors credit they have avoided this in a masterful manner They have given an all round and living picture of the topic in a sophisticated and undogmatic manner The reader obtains a good appreciation for the area s complicated and multi faceted nature Textbooks with these qualities—concise lucid and covering the essential issues—are needed for the education of medical students Such a book has been lacking in child psychiatry No criticism can be directed against the factual content of the book Nevertheless I would suggest supplementing some of the content in a later edition The authors discuss the topic from biological psychological and social aspects This is in my opinion not sufficient today We live in a changing society and this is something which they should have discussed The increasing incidence of child abuse child delinquency and criminality drug abuse suicidal acts etc is at present apparent in all countries in western civilisation These conditions must in all likelihood be associated with the society we live in Greater demands are likely placed on parents today than earlier not the least as a consequence of the increasing alienation in society with deficient support from relatives, neighbors etc Parental alcohol abuse is a factor which appears to be especially harmful to children

Another area which has been missed in the book concerns the extensive problems of a child psychiatric nature which pediatricians encounter in their work A majority of the cases which come to the child psychiatrist have pre in child psychiatry and is not uncommonly precipitated by alcohol or drug abuse or serious psychic trauma The appearance of schizophreniform symptoms should not lead to confusing this psychosis with schizophrenia I also missed a section on acute child psychiatry, which requires more and more of the child psychiatrist s time It is important for the physician to-be to realize that the frequent acute cases place great demands on the child psychiatrist and require great professional skill

In spite of these reservations I would nevertheless warmly recommend this book, and feel it should be included as required literature in a course in child psychiatry He who has read this book will have received a good foundation for his subsequent practice as a physician Many readers may also be stimulated to continue their study of the topic

Ingvar Nylander

S E Gerber & G T Mencher (eds) *Early diagnosis of hearing loss* 377 pp illus Grune & Stratton New York 1978 No price given ISBN 0-8089-1153-8

The importance of early detection of hearing loss in children is unquestionable This book is a report on a conference on Early Diagnosis of Hearing Loss held in Saskatoon in May 1978 The conference was especially devoted to methods for confirmation of the presence and degree of hearing loss within the first six months of life as rapidly and as economically as possible'

To the pediatrician the criterion for identifying a newborn at risk for hearing impairment must be known A high risk register should be used and the most important risk factors are discussed A prospective study shows that asphyxiated infants make up a significant proportion of the deaf children Other important etiologies are congenital rubella, familial deafness and bacterial meningitis in the neonatal period

Topographical signs of congenital hearing loss are systematically described and abnormal signs on the pinna that should lead one to suspect a co-existing hearing loss are emphasized

Why early diagnosis? According to Hallowel Davis the necessity of stimulation for the full development of a sensory system is generally accepted This means that amplification with a hearing aid should be used as early as possible once hearing loss is confirmed Appropriate amplification requires knowledge of hearing thresholds, and the use of electric response audiometry is necessary to find the hearing thresholds in children less than two years of age

The principal methods are electrocochleography (ECochG) where an intratympanic electrode is used and the brain stem responses (BSER) obtained by surface electrodes Since most hearing losses are due to periph

DISCUSSION

A striking similarity exists between the physical and initial laboratory findings of this infant with congenital nephrotic syndrome and those expected in infants with hypothyroidism These include wide sutures with a large patent posterior fontanelle, coarse hair, depressed nasal bridge, dry skin, abdominal distention with an umbilical hernia, and edema (6, 8) In addition, although not seen in this case, respiratory distress, poor feeding, and decreased activity have been reported as features of both syndromes (8) She had a low T_4 and high normal TSH suggestive of hypothyroidism, although the TSH is usually much higher in congenital hypothyroidism (4, 7) These findings, combined with evidence of euthyroid function as indicated by the availability of thyroxine, the free T_4, suggested TBG deficiency, (3, 4) which was subsequently confirmed However, congenital TBG deficiency does not explain the edema Consequent examination of the urine revealed marked proteinuria and microscopic hematuria with the resultant hypoalbuminemia and hypercholesterolemia characteristic of nephrosis Renal biopsy was compatible with the diagnosis of congenital nephrotic syndrome Urinary losses of TBG and thyroxine (1, 5) probably led to the low serum TBG and low T_4

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In summary, we have observed an unusual presentation of congenital nephrosis and we wish to alert physicians to the striking clinical and laboratory similarities between this and hypothyroidism, and stress the importance of ruling out proteinuria and measuring serum TBG whenever any question arises The importance of considering this diagnosis is especially important in areas of the world such as Finland, where the estimated incidence of the congenital nephrotic syndrome approaches one-third that of congenital hypothyroidism and is only slightly less frequent than the occurrence of congenital TBG deficiency (2, 3, 4, 6)

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Submitted Febr 18 1980
Accepted April 3 1980

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Acta Paediatr Scand 69 709–715, 1980

AUTOANTIBODIES TO TAMM HORSFALL GLYCOPROTEIN IN CHILDREN WITH RENAL DAMAGE ASSOCIATED WITH URINARY TRACT INFECTIONS

A FASTH J BJURE M HELLSTRÖM B JACOBSSON and U JODAL

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ABSTRACT Fasth A, Bjure
ments of Pediatrics Clinical
Radiology) Autoantibodies to
associated with urinary tract
bodies to the Tamm-Horsfall (TH) protein were analyzed in sera from 116 patients with pyelonephritis The increases in antibody levels were limited in 23 patients with radiological detected renal damage during 31 attacks of acute pyelonephritis 8 children with abnormally low total and/or unilateral ^{5} Cr EDTA clearance had significantly lower IgG antibody levels to TH protein than 14 children with normal clearance rate All 61 children with renal damage had significantly low IgG, IgA and IgM antibody levels to TH protein 6 months after last infection as compared to the reference group For IgG antibodies, the mean was well below −2 S D 12 children with increased serum creatinine had significantly lower IgG antibody levels than those with normal creatinine No correlation was found between antibody levels and vesico ureteric reflux In contrast, 55 children with no renal damage still had significantly increased IgG and IgA antibody levels to TH protein 6 months after the infection The mechanism for the low antibody levels was discussed and it was concluded that patients with urinary tract infection and low IgG antibody levels to TH protein were at risk for renal damage and should be radiologically examined

KEY WORDS *Autoantibodies Tamm Horsfall protein renal damage, intravenous urography* ^{5} Cr EDTA clearance

Many investigations and follow up studies have been performed on patients with urinary tract infection (UTI) as this disease presents a substantial risk for renal damage and functional impairment In the epidemiological studies on children of Winberg et al (20) 5% of the girls and 13% of the boys followed from their first symptomatic UTI had or developed renal scarring as judged from intravenous urography (IVU) In spite of careful treatment and follow up the renal lesions were progressive for 90% of the girls and 50% of the boys (3 21) There is obviously a need to identify those patients who are at risk for renal damage but there is unfortunately no simple and reliable test for this or for detecting those with progressive deterioration of renal function

Previous studies have shown that all humans have serum autoantibodies to the Tamm Horsfall (TH) kidney protein (7, 10) During attacks of acute pyelonephritis, an increase in IgG and IgA specific antibodies has been observed (7 10 15) This response was most marked in patients who, on the following x ray, showed a *vesico ureteric reflux* (10) In contrast, girls with renal damage but no UTI at the time of testing were found to have depressed serum IgG levels to the Th protein (10)

In the study presented here these findings have been further elucidated by repeated serum sampling from children with damaged kidneys, in an attempt to identify the serological response to *E coli* and the TH *protein* The results have been related to parameters of renal damage and function

eral (cochlear) impairment, it might be wise to investigate directly the auditory periphery by means of ECochG. The pioneer on clinical electrocochleography, dr J.-M. Aran, recommends that in all cases presenting with ECochG responses, the auditory pathways be tested too. This can be done using BSER with surface electrodes. Dr Galambos has used BSER as a screening procedure in the normal newborn nursery. In his hands BSER readily identifies the baby with a significant hearing loss at the earliest possible moment in his life. Since BSER is a non-invasive method it seems to be the most suitable method for neurophysiological screening of infants at risk.

From this book the pediatrician can readily learn what is needed for proper selection of infants at risk for hearing impairment. Most audiologists will probably be confused by the contradictory opinions on the value of the different diagnostic techniques. This is a necessary consequence of putting together about fifteen lectures by authors with more or less differing opinions.

Sten Harris

C. G. Geary *Aplastic Anaemia*. 249 pp., illust. Baillière Tindall, London 1979. ISBN 0-7020-0698-X. $17.85.

Since the first description of aplastic anaemia by Ehrlich appeared in 1888 much information has been gained about the disease. Especially in the last ten years techniques in immunology and tissue culture and improved results of treatment have increased our understanding of the disease and its underlying marrow defect.

This book is a comprehensive monograph on aplastic anaemia and gives detailed and fascinating information about pathophysiology, dyserythropoiesis and drug mechanisms in marrow hypoplasia, laboratory aspects and clinical features of aplastic anaemia and its treatment: conservative management as well as bone marrow transplantation and the use of antilymphocyte globulin. Separate chapters deal with aplastic anaemia in childhood, red cell aplasia and the aplasia–leukemia syndrome.

The authors are most experienced experimental and clinical haematologists with in many ways well documented expertise in treating the disease in both [illegible]

[illegible] to all who are involved in the diagnosis and treatment of aplastic anaemia.

Anders Kreuger

B. F. Trump & R. T. Jones *Diagnostic electron microscopy*. John Wiley & Sons, New York, Chichester, Brisbane, Toronto 1979. 401 pp., illus. Price not given. ISBN 0-471-89196-7.

Veterans Administration in the US has a large program for diagnostic electron microscopy units all over the country. The mean number of specimens analysed per year was 473 in 1977. The present book Diagnostic electron microscopy, volume 2, edited by Benjamin F. Trump and Raymond T. Jones describes the experience from the diagnostic work in the Veterans Administration. The book consists of several chapters, written by different authors and deals with liver pathology, pathology of the hematopoietic system, pathology in ophthalmology, urology, gynecology, and neurology. The book emphasizes the importance of electron microscopy in diagnostic pathology and it clearly shows the usefulness of the electron microscope for correct diagnosis of diseases which cause subcellular changes below the resolution of the light microscope.

Unfortunately, several of the contributors of this work describe changes of subcellular components as pathological changes specific for the underlying disease although the electron microscopical features of these changes clearly are preservation artefacts as shown by their illustrations. This is a major draw-back of the volume and it can therefore not be uncritically used as a reference book. However, if the reader is familiar with artefacts produced by the preservation of tissue for electron microscopy the book, in selected parts, may be useful in diagnostic pathology.

Lars Larsson

ANNOUNCEMENT

INTERNATIONAL SYMPOSIUM ON HUMAN MILK

The 1st International Symposium on Human Milk will be held May 18–21, 1981 in Hradec Kralove, Czechoslovakia. The symposium is organized by Tissue Bank, Teaching Hospital Hradec Kralové under the auspicies of C I Commission of International Institute of Refrigeration (Paris) and Low Temperature Biology Section of the Czechoslovak Academy of Sciences. It will include all problems dealing with human milk, especially human milk banking. Secretary of the symposium: Ing. Lubomir Vavra, Fakultni nemocnice KUNZ, Tkáňová ustredna, 500 36 Hradec Kralove, Czechoslovakia will give further information.

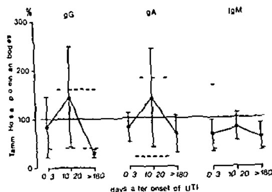


Fig 1 IgG IgA and IgM antibody levels to Tamm Horsfall protein during 31 attacks of acute pyelonephritis in *children with renal damage* judged by IVU The values given are the mean ± 1 S D The mean and ± 2 S D of the reference material are indicated The antibody levels are expressed as a percentage of the reference group The increases for IgG and IgA antibodies from 0–3 days to 10–20 days are significant ($p<0\ 02$ and $p<0\ 01$ respectively) All mean values at >180 days are significantly ($p<0\ 001$) depressed compared to the reference group

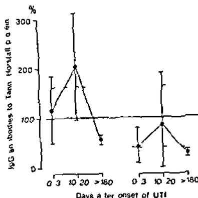


Fig 2 IgG antibody levels to Tamm Horsfall protein during acute pyelonephritis in 22 children who had Cr *EDTA clearance and radiorenogram performed* At the left is illustrated the response in 14 children with normal clearance and at the right that of 8 children with abnormally low total or unilateral clearance The differences between the mean levels of the two groups at onset and 10–20 days later are significant ($p<0\ 01$)

EDTA clearance and side distribution of kidney function by renography (9) The single injection ^{5} Cr EDTA total plasma clearance determination was used for clearance measurements (2) The renographic examination was performed in a standardized manner as described by Bjure et al (1) and unilateral kidney function was calculated (11)

Tamm Horsfall protein was prepared as earlier described (8)

For the analysis of *antibodies to Tamm Horsfall protein* was used the enzymelinked immunoassay (ELISA) of Engwall & Perlman (5) with earlier described modifications (7) The tubes were coated with 10 mg/l TH protein The conjugates were prepared using anti IgG anti IgA or anti IgM from Dakopatts A/S Copenhagen Denmark and alakaline phosphatase from Sigma Chem Co St Louis USA The absorbance was read at 405 mm and converted to that corresponding to a reaction time of 100 min The readings were expressed as percent of the mean of the reference material i e 100% mean of the references

The relative avidity of the IgG antibodies to TH protein was tested by use of the ELISA (18) Sixty sera from patients and references with varying amounts of IgG antibodies to the TH protein were incubated for only 30 min in tubes coated with TH protein and the values found were expressed in percent of those recognized after the usual 5 hours incubation i e a high percentage and a rapid binding of the antibody to antigen corresponded to a high avidity (18)

Antibodies to a pool of E coli O antigens common in UTI (0/1 0/2 0/4 0/6 0/7 0/8 0/18 0/75) were analyzed using the indirect hemagglutination technique (IHA) (13) The antigens were heat extracted at 100°C for 2 hours and the supernatants were used (13) The sera were ana

Table 3 *Analysis of autoantibodies to Tamm Horsfall protein in patients with previous acute bacterial pyelonephritis but no infection during the previous six months*

The values given are mean + 1 S D and they are expressed as a percentage of the mean of the reference group IVU = intravenous urography

		Autoantibodies to Tamm Horsfall protein		
Group	No	IgG	IgA	IgM
Renal damage on IVU	40	79 0±10 1[a]	67 6±39 8[a]	66 1±25 1[a]
Normal IVU	55	151 1±62 5[b]	155 7±67 1[b]	106 8±78 7
Controls	70	100±30	100±40	100±32 5

[a] $p<0\ 001$ i e significantly *depressed* levels compared to the controls
[b] $p<0\ 001$ i e significantly *increased* levels compared to the controls

Table 1. *Patients with renal damage judged by i.v. urography and suffering from acute bacterial pyelonephritis*

Group	No of patients	No of attacks of pyelonephritis	Mean age, y (range)	Intravenous urography: Normal	Intravenous urography: Renal damage
A	23	31	3 6 (0 5–11)	0	23

MATERIAL AND METHODS

Patients A total of 116 patients with actual or previous acute bacterial pyelonephritis were investigated as part of regular examinations at the Children's Hospital, Goteborg All had intravenous urography (IVU) and most of them also voiding cystourethrography (VCU) performed

The patients were divided into three groups

A 19 girls and 4 boys who had or later developed renal damage were followed during one or several attacks of acute pyelonephritis (Table 1) Serum was obtained within the first days after onset, after 10–20 days and 6 months or more after the last infection 31 UTI, 12 primary and 19 recurrent, were studied 12 of the children, 9 girls and 3 boys, were also included in group B

B 50 children, 39 girls and 11 boys with renal damage were investigated 6 months or more after the latest UTI (Table 2)

C 55 children 49 girls and 6 boys, with previous UTI and normal IVU were investigated 6 months or more after the latest infection (Table 2)

Reference material 70 children and adults previously described were used as controls (7)

Serum samples were stored at −20°C until analyzed

Diagnostic criteria were those employed in earlier studies (7, 14, 16) Growth of ≥10⁵ bacteria/ml midstream urine or any amount in urine obtained by suprapubic bladder puncture was required for a diagnosis of UTI In addition the following criteria were used for a diagnosis of acute pyelonephritis fever >38 5 and at least two positive tests of ESR (≥25 mm/hour) and CRP (≥20 mg/l) and a transient decrease of renal concentrating capacity (<815 mOsm/kg) To be judged UTI free the patients were required not to have had bacteriuria during the previous 6 months

Radiological studies Intravenous urography (IVU) The patients were examined 1–3 months after primary and 2–8 months after recurrent infection They were investigated supine with a film to focus distance of 1 00 m The contrast medium used was Isopaque 350 g/l The dosage varied with body weight as follows <5 kg 4 ml/kg, 5–10 kg 20 ml, 10–20 kg 30 ml and >20 kg 40 ml The parenchymal thickness at the upper and lower pole and at the lateral aspect of the kidney was measured as described by Claesson et al (4) Renal damage was defined as parenchymal thickness below −2 S D at any aspect, regardless of whether it was associated with scarring or dilatation

Voiding cystourethrography (VCU) Reflex was graded slightly modified, according to Heikel & Parkulainen (12) grade I—filling of ureter only grade II—filling of ureter and renal pelvis without dilatation grade III—dilatation in ureter and renal pelvis no or slight dilatation in calyces Grade IV—dilatation in ureter and renal pelvis dilatation in calyces with blunting Grade V—gros dilatation in ureter and pelvis general clubbing of calyces

Evaluation of total and unilateral kidney glomerular filtration In 22 of the children in group A the total and unilateral kidney filtration 3–18 months after the acute pyelenephritis was estimated by a combination of ⁵¹Cr

Table 2. *Patients with previous acute bacterial pyelonephritis but no UTI for the 6 months preceding examination*

Group	No	Age,[a] y (range)	Serum creatinine[a] mMol/l (median)	Renal concentrating capacity[a] (mOsm/l)	Intravenous urography: Normal	Intravenous urography: Renal Damage	Voiding cystourethrography: Reflux grade 0–1	Voiding cystourethrography: Reflux grade 2	Voiding cystourethrography: Reflux grade ≥3	Voiding cystourethrography: No investigation[b]
B	50	7 8±4 9 (1–17)	112±173[c] (76)	739±216[d]	0	50	13	9	12	16
C	55	8 0±4 3 (1–18)	62±11[c] (60)	991+79[d]	55	0	50	5	0	0

[a] The values given are mean ± 1 S D
[b] [illegible]
[c] [illegible]
[d] [illegible]

concentrating capacity and TH protein antibodies

The depressed values of IgG autoantibodies to TH protein found in sera from UTI free children with renal damage were compared to the values found in *55 UTI free children with previous UTI and normal IVU* Six months after the infection these 55 children still had significantly increased IgG and IgA, but not IgM antibody levels to TH protein compared to the controls The values showed great variation with several values above +2 S D , but no IgG value below −1 2 S D of the reference group The IgM antibody levels to TH protein showed almost the same mean and standard deviation as the controls

Relative avidity of the IgG antibodies against TH protein

The binding rate of the IgG antibodies to the TH antigen on the tube walls in the ELISA was found to be closely related to the levels of IgG antibody (Fig 2) The sera with high levels of IgG antibodies to the TH protein bound very quickly, the levels observed at 30 min were almost the same as those found after 5 hours The sera with very low amounts of specific antibodies i e sera from patients with renal damage, showed a slow binding rate, however and required a total of 5 hours to obtain maximal binding to the antigen

DISCUSSION

The present communication confirms the findings of low levels of antibody to TH protein in sera from patients with kidney damage (6, 7) The specific IgG levels of the majority of patients were below −2 S D of the reference material and none had IgG antibody levels to TH protein above −1 5 S D The possibility that all patients suffering from attacks of acute pyelonephritis demonstrate low antibody levels to TH protein was excluded In contrast, those patients with no radiologic evidence of renal damage had increased IgA and IgG antibody levels to TH protein even 6 months after the attack of UTI The IgG levels of antibodies to TH protein in this group showed a greater variation, but no patient had values below −1 2 S D The patient with depressed IgG antibody levels to TH protein and a history of UTI should be considered to be at risk for having kidney damage and should thus be radiologically examined

Those patients with renal damage were found to be poor producers of antibodies to TH protein during attacks of acute pyelonephritis In fact, the mean maximal IgA and IgG antibody levels to TH protein did not exceed +2 S D of the controls This is in agreement with the findings in three adult female patients with renal damage These women, 14 days after onset of acute pyelonephritis, had IgG antibodies to the TH protein below +2 S D of the reference material (20) The findings contradict previous reports, however, where girls with acute pyelonephritis showed a marked increase in their IgG and IgA antibody levels (7, 10, 15) In fact, the highest levels in these studies were noted among those with vesicoureteric reflux, but only two of those 18 patients had renal damage

A correlation between abnormally high serum creatinine levels and low IgG anti-TH antibodies has been demonstrated in an adult population of patients with severe kidney damage (6) The children in this study also showed a relationship between serum creatinine and IgG antibodies to TH protein, as the 12 patients with increased serum creatinine had significantly lower antibody levels than those with normal creatinine

It is possible that during an acute attack of pyelonephritis there is a leak of TH glycoprotein into the circulation and hence a stimulation of antibody formation A vesicoureteric reflux would increase the introduction of TH protein in the interstitial tissue and the circulation, and further stimulate antibody formation It is more difficult to explain the very low IgG anti-TH protein levels in UTI free periods in patients with renal scarring A possible mechanism is adsorption or consumption of the

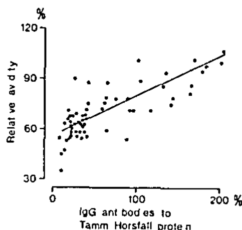


Fig 3 Relative avidity of IgG antibodies to Tamm Horsfall protein in 60 sera from 30 patients with actual or previous acute *pyelonephritis* as well as *from 30* of the references The IgG antibody levels are expressed as a percentage of the mean of the reference and the relative avidity as 100 × antibody levels obtained in sera incubated 30 minutes/antibody levels in sera incubated 5 hours (see text) Regression analysis yielded $y=54.22-0.25x$ $r=0.884$ $p<0.001$

lysed before and after mercaptoethanol (ME) treatment The titers found were expressed as $\log_2$

The *serum creatinine* was analysed according to the method of Jaffe in an automated system (Greiner Selective Analyser Langental Switzerland)

Statistical procedures The Student *t* test was used to compare the means Statistical significance was defined as $p<0.05$

RESULTS

Children with renal damage and acute pyelonephritis (group A)

The mean antibody response to the TH protein was limited during the 31 attacks of acute bacterial pyelonephritis *12* primary and *19* recurrences among the 23 children with renal damage (Fig 1) The mean IgG and IgA, but not IgM, antibody levels increased significantly, but never exceeded +2 S D of the reference group The antibody levels noted in serum from the children with primary infections were higher than those in children with recurrences, though the difference was not statistically significant Six months after the acute pyelonephritis the means of the IgG, IgA and IgM antibody levels to TH protein were significantly lower than those of reference group The mean for IgG autoantibodies was well below −2 S D of the reference group

Radiorenogram and ^{51}Cr EDTA clearance were performed in 22 patients and the total and unilateral glomerular filtration estimated Eight children, all with recurrent pyelonephritis, had abnormally low total and/or unilateral filtration rates These 8 children also had, in comparison to the 13 with normal filtration, *significantly lower mean IgG* antibody levels to TH protein, both at the onset of the infection and 10–20 days later (Fig 2) Six of the children had no IgG antibody increase as compared to only one of the children with normal filtration rate

The IHA titres to the pool of O antigens *were measured in 26 of the 31 acute* attacks of pyelonephritis Half of the UTI were caused by *E coli* belonging to the pool, and 9 of the children showed a significant increase, i e 2 or more titre steps Furthermore, no correlation was found between the serological response to O-antigen and the TH protein

Children with previous acute pyelonephritis but no recent UTI (group B and C)

The 50 children who had renal damage and previous UTI but who were UTI free at time of testing and for the preceeding 6 months had significantly depressed levels of IgG and IgA as well as of IgM antibodies to TH protein, compared to the reference group (Table 3) The mean level of IgG autoantibodies was well below −2 S D Ten of the 58 patients had IgG levels between −2 and −1 5 S D of the reference group and none had levels above −1 5 S D

Twelve children had serum creatinine levels above the normal range for their age These children also had significantly lower IgG antibody levels to TH protein compared to the children with a normal serum creatinine ($p<0.001$)

No correlation was found between the degree of vesico ureteric *reflux and IgG anti*bodies to the TH protein, nor between kidney

21 Winberg J Claesson I, Jacobsson B Jodal U & Peterson H Renal growth after acute pyelonephritis in childhood An epidemiological approach *In* C J Hodson & P Kincaid Smith (ed) *Reflux nephropathy* Masson Publishing Co, New York 1979, p 309
22 Zager R A Cotran R S & Hoyer J R Pathological localization of Tamm Horsfall protein in interstitial deposits in renal disease *Lab Invest 38* 52 1978

Submitted March 21, 1980
Accepted May 27, 1980

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produced antibodies in the disased kidneys Such an explanation may be supported by the recent findings of several independent groups, who found that the periodic Schiff positive deposits often observed in damaged kidneys were composed of TH protein (23, 24, 25) Solez & Heptinstall (24) even demonstrate polypoidal projections into the kidney veins, which stained positively for TH protein Such deposits of antigen in the tubulointerstitial tissue could also explain why the measured antibody response in patients with previous renal damage was low compared to patients with normal kidneys

Indirect evidence of consumption or adsorption was given by the low avidity of IgG antibodies found in sera from the patients with renal damage The antigen stimulation during an attack of acute pyelonephritis should then increase the avidity of the antibodies formed just as is found in other infectious diseases An alternate interpretation could be that the low avidity was due to an immunosuppressive effect of TH protein and development of tolerance This, however, seems less likely as some of the patients had clearly increased IgG anti-TH protein antibody levels with high avidity two weeks after the onset of the infection, but, in spite of this, they showed low antibody levels with low avidity 6 months later

ACKNOWLEDGEMENTS

This work was given financial support by the Swedish Medical Research Council (No 215) and the Medical Faculty University of Goteborg Goteborg Sweden Thanks are due to Ingela Carlsson for skillful technical assistance

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Submitted March 21 1980
Accepted May 27, 1980

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Acta Paediatr Scand 69 717–722, 1980

RESPIRATORY SYNCYTIAL VIRUS INFECTIONS IN OSLO 1972–1978

I Virological and Epidemiological Studies

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ABSTRACT Ørstavik, I , Carlsen K H and Halvorsen, K (Microbiological Laboratory, Ullevaal Hospital, and the Departments of Paediatrics, Ullevaal and Aker Hospital, Oslo, Norway) Respiratory syncytial virus infections in Oslo 1972–1978 I Virological and epi [illegible]

disease [illegible] 70 % As a rule RSV infections occurred in distinct outbreaks in late autumn and winter together with a marked increase in the number of children admitted to hospital with acute lower respiratory tract disease The incidence in children less than 1 year of age was about 10/1 000 children/year Spread of the infection from one end of the city to the other was discernible in 2 outbreaks More children than expected with siblings were admitted to hospital because of RSV infection, but no correlation could be observed with some other socioeconomic factors A negative correlation was observed between RSV disease and mean air temperature and hours of sunshine per month Rapid immunofluorescence diagnosis of RSV on cells from nasopharyngeal secretions was adopted and became comparable to cell culture technique The rapid method has become an important adjunct to the clinical management of these patients and the method will form the basis for further epidemiological studies

KEY WORDS Respiratory syncytial virus, epidemiology rapid virus diagnosis

Respiratory syncytial virus (RSV) is considered the most important respiratory pathogen in infants and small children (4) and winter outbreaks have been reported from different parts of the world (1, 3, 9 11) Recent studies from Great Britain have suggested that the incidence of severe illness caused by the virus may vary considerably in different locations (16 17)

In Oslo the first outbreak of RSV bronchiolitis was studied virologically by Ulstrup in 1962 (18) From the autumn of 1972, a more complete virological surveillance has been attempted on children admitted to the paediatric departments of The Oslo City Hospitals with acute respiratory infections A report of the RSV epidemic of 1972/1973 has previously been published in Norwegian (14) In the present report we will describe our virological and epidemiological findings for the period from 1972 to 1978 in an attempt to assess the virological methods, the incidence of disease, and factors that may affect the epidemiology of RSV infections The clinical findings together with the results of other laboratory studies will be the subject of a following paper (2)

MATERIAL AND METHODS

Population and patients

During the study period from November 1972 through June 1978 the total population of Oslo varied between 474 208 and 459 100 inhabitants The Paediatric Department of Ullevaal Hospital was the only paediatric department for the city until the summer of 1975 when another department at Aker Hospital was opened Patients in Ullevaal Hospital were studied during the entire period patients from Aker Hospital from the autumn of 1976

Table 1. *Diagnosis of RSV infections and virus surveillance rates during 6 epidemic years*

Epidemic year	Surveillance	No. of patients diagnosed by: Virus identification/isolation	Antibody titre rise only (CFT)	Total
1972/73	77%	78 (55/77)[a]	19	97
1973/74	68%	29 (10/28)	5	34
1974/75	69%	95 (49/93)	13	108
1975/76	70%	38 (29/37)	7	45
1976/77	62%	76 (66/72)	4	80
1977/78	73%	93 (76/89)	7	100
Total	70%	409 (285/396)	55	464

[a] Numerator = No. of specimens RSV identified by rapid immunofluorescence technique. Denominator = No. of specimens tested both by rapid immunofluorescence technique and by cell cultures.

Infants and children admitted to hospital with symptoms of acute respiratory infections, also patients with acute asthma and febrile convulsions, were examined virologically. Retrospectively, the clinical and laboratory findings of patients with RSV infections were collected from their clinical records.

An 'epidemic year' for RSV infections is defined as the 12 months from July through June the next year.

Virological methods

Nasopharyngeal secretions were usually collected on the morning following the patient's admission to the hospital. Cells from the specimens were examined for RSV antigens by a rapid, indirect immunofluorescent antibody technique, as described by Gardner & McQuillin (6). Two RSV immune sera were used for the immunofluorescence test. During the first half of the study period a serum prepared in guinea pigs by intranasal installation of virus and during the second half an immune serum against purified nucleocapsid of RSV prepared in a rabbit by J. Ulstrup, Ullevaal Hospital. Specimens stained by guinea pig immune serum and conjugate were often difficult to read due to unspecific staining, whereas the rabbit immune serum was highly specific. Secretions were also inoculated into cell cultures of human fibroblasts, HeLa, HEp2-, and primary monkey kidney cells for virus isolation. Acute and convalescent (usually 2–3 weeks later) serum specimens were tested for RSV antibodies by the complement fixation test (CFT) as described by Grist et al. (8). The initial dilution of serum tested was 1:4 and for infants less than 1 year, the amount of antigen was increased to 8–16 doses in the CFT. RSV infection was diagnosed by identification/isolation of RSV from the secretion and/or by finding a significant antibody titre rise (at least 4 fold) from acute to convalescent serum.

Statistical methods

The chi square, the Wilcoxon Mann-Witney (W.M.W) and the Kendall rank correlation coefficient tests were used.

Table 2. *Virological findings in 184 RSV infected patients examined both by virus identification/isolation (Isol.) and by serology (CFT)*

Result of test: Isol.	CFT	Age group (years): 0–0.5	0.5–1	1–2	>2	Total
Pos	Pos	10	37	15	18	80
Neg	Pos	3	3	3	16	25
Pos	Neg	49	20	6	4	79
Total		62	60	24	38	184

RESULTS

Virological surveillance

Specimens for virological examination—nasopharyngeal secretions and/or paired serum samples—were collected from approximately 70% of the patients admitted with symptoms of acute respiratory infection (Table 1).

Virological findings

RSV infection was diagnosed and data collected from a total of 464 patients, of whom 422 were from Ullevaal Hospital and 42 from Aker Hospital (Table 1).

More cases were diagnosed by the rapid immunofluorescence method during the latter part of the study. Of the 161 nasopharyngeal secretions examined both by the rapid test and

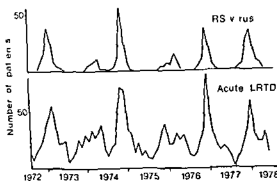


Fig. 1 Total number of RSV infections per month and number of patients admitted to hospital with acute lower respiratory tract disease per month

by cell culture during the last two epidemic years 87% were identified by the rapid test and 94% by cell culture, whereas 81% were positive by both methods

Nasopharyngeal secretions and paired serum samples were examined from 184 of the patients and a comparison of the results is shown in Table 2 Among the 63 patients less than 6 months of age a rise in antibody titre could be detected in only 13 In patients more than 2 years of age CFT on paired serum samples was more efficient than virus identification/isolation

Seven of the 464 patients got their RSV infection as the result of a hospital cross infection and these patients are not included in the epidemiological data which follow

Temporal distribution of illness due to RSV

Distinct outbreaks of RSV infection occurred during the epidemic years of 1972/1973, 1974/1975 1976/1977 and 1977/1978 During these outbreaks a sharp rise in the total number of admissions of children with acute lower respiratory tract disease was observed (Fig 1) No abrupt rise in the total number of admissions occurred during the winters of 1973/1974 and 1975/1976, and the number of RSV infections during these winters were less and did not occur in distinct outbreaks The patients admitted to The Paediatric Department of Aker Hospital during the epidemic year of 1975/1976 were not examined virologicall[y] This period, therefore, is not included in th[e] epidemiological results which follow

Incidence of RSV disease

Only minor variations were observed in t[he] incidences of hospitalized children with RS[V] infection for four of the five epidemic yea[rs] (Table 3) The incidences for 1973/1974 we[re] only about half of those of the other yea[rs] No calculations were made for age grou[ps] over 2 years where the number of cases w[as] very small

Socioeconomic factors and geographical spread of infection

We did not make a socioeconomic classifi[ca]tion of the parents of the individual patie[nts] but a correlation test was made between [the] incidence of cases in a township and the av[er]age income in the township of the patie[nt's] residence (Oslo is divided in 35 townshi[ps]) We found no correlation between these pa[ra]meters

The percentage of patients who had a si[ngle] parent (11 7) was almost the same as for [the] total child population less than 3 years of [age] (13 1)

The proportion of children with RSV in[fec]tion who had siblings was greater than exp[ect]ed from the population statistics (58%, [chi] square = 13 5, $p<0\ 001$)

During two of the epidemic years, the I

Table 3 *Incidence of RSV infection as d[iag]nosed in hospitalized children in Oslo 1[972–]1978*

Epidemic	No of cases per 1 000 childr[en] in age groups	
	0–1 y	1–2 y
1972/73	11 1	5 1
1973/74	5 8	1 4
1974/75	11 0	3 4
1976/77	9 4	3 6
1977/78	10 2	3 4

infection was found to be spreading from one end of the twon towards the other (from east to west)

Meteorological factors

We found a statistical significant, negative correlation ($p<0.05$) between mean daily temperature per month, hours of sunshine per month, and the occurrence of RSV infection per month No significant correlation was observed between the mean of rainfall/snow per month, the average humidity per month, and cases of RSV infection

A statistically significant, positive correlation ($p<0.001$) was observed between the number of cases per month and the air pollution measured by the content of SO_2 in the air

DISCUSSION

Virus identification by rapid immunofluorescence or virus isolation in cell culture was found to be superior to serological diagnosis of RSV infection in the important age group less than 1 year Others have also found the CFT to be of low sensitivity in small children (1, 15) Increasing emphasis was therefore laid on virus identification/isolation

The sensitivity of the rapid immunofluorescence technique was increased during the study period (Table 1), partly due to improvements in reagents, but also because of an increased awareness of the need for standardiza tion of sampling, transport and preparation of the specimens A positive result was usually obtained on the same day as the specimen was taken The rapid technique led to a close cooperation between the clinician and the virus laboratory which helped to maintain the rate of virological surveillance

During the five epidemic years of com prehensive surveillance, sharp outbreaks of RSV infection occurred during four, with epi demic peaks in January, November, Decem ber, and January, respectively A typical epi demic lasted for about 5 months and had reached its peak by the second month RSV infections were almost absent in the summer and during the entire study period it was only detected once in each of the months from June through September Others have reported a similar temporal distribution of RSV infections (1, 4, 10, 12)

The seasonal variations of RSV infections may be due to meteorological factors Martin and coworkers found RSV epidemics to be inversely correlated to middle temperature and to hours of sunshine (12) An inverse cor relation was also observed between these meteorological parameters and the number of RSV infections in the present study, as well as a positive correlation to air pollution We were, however, unable to detect meteorologi cal data that could explain the variations in the time of onset of epidemics and the small num ber of cases during the epidemic year of 1973/1974 More cases of RSV infection did not occur in areas of the town where the air was more heavily polluted than in some of the less polluted areas These findings suggest that the correlations may not imply a cause and effect relationship

The observed incidences of RSV infection during four of the five epidemic years of the present study were intermediate to the inci dences reported from Great Britain for urban and for industrial areas (16, 17) A lower in cidence for RSV disease was suggested in a study from Washington, D C (10) Probably more children were admitted to our hospitals each year because of RSV infection than the number diagnosed in the present study Some may not have been detected by the virological examination and some may not have been examined virologically at all An unknown number of patients with acute laryngitis prob ably due to RSV were treated in the ear nose-throat department of the Oslo City Hospitals without being examined virologically

Data from the present study suggest a tem poral spread throughout the town of RSV in fection during some of the epidemics Others have found school children to be important as

transmitters of RSV infection (5, 13). In Oslo the children of this age group spend most of their time within the neighbourhood of their homes, and this may explain why a regular pattern of geographical distribution of cases was discernible

We were unable to demonstrate an association between socioeconomic conditions and an increased number of RSV infections admitted to hospital when we considered such factors as the average income of the patient's township and the percentage of patients with a single parent Others have found a positive correlation between poor socioeconomic conditions and an increased number of RSV cases (7, 17) However, the socioeconomic conditions in our study and in the studies mentioned above may not be comparable Children with siblings were more likely to be admitted to hospital than those without This probably reflects the importance of siblings in the transmission of the infection (16)

The present study has demonstrated the unique importance of RSV infection as a cause of acute lower respiratory tract disease requiring hospital admission in Oslo As a result of the study the rapid immunofluorescence technique for diagnosing RSV infection was adopted and developed by the virus laboratory. The main advantage of this method is that it may aid the clinician in his management of the individual patient (2) The increased demand for virological diagnosis that has been the result of this development will form the basis for further epidemiological studies of RSV infections in our area

ACKNOWLEDGEMENTS

Statistical and meteorological data were obtained from The Oslo City Office for Statistics and The Norwegian Meteorological Institute We thank J Holme and E Fonstelien Ullevaal Hospital for statistical advice, and T Gedde Dahl National Institute for Public Health Oslo for valuable discussions Financial support was received from Oslo kommunes medisinsk videnskapelige

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Submitted Febr 27 1980
Accepted May 22 1980

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Acta Paediatr Scand 69 723–729, 1980

RESPIRATORY SYNCYTIAL VIRUS INFECTIONS IN OSLO 1972–1978

II *Clinical and Laboratory Studies*

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with bronchiolitis and pneumonia Rapid virus diagnosis by immunofluo... decreasing utilisation of antibiotics in patients with bronchiolitis and to a shorter stay in hospital The value of antibiotics in these infections is discussed.

KEY WORDS Respiratory syncytial virus, bronchiolitis, pneumonia, otitis media, rapid virus diagnosis, antibiotic treatment

Respiratory syncytial virus (RSV) is a major cause of illness in childhood and infancy Some studies indicate that 70–80% of cases of bronchiolitis in children below the age of one year are caused by RSV (15) The morbidity is considerable, and RSV infections in this age group often require admission of the patient to the hospital Usually the mortality rate is reported to be low (14, 15), but recently an outbreak seems to have caused a number of deaths among the affected children (1, 10)

This paper concerns 422 children admitted to the Paediatric Department, Ulleval Hospital Oslo during 6 outbreaks of RSV infections during the winter seasons 1972–73 to 1977–78 Extensive virological surveillance was performed The epidemiological and virological findings are presented elsewhere (13) In the present study clinical aspects of illness are investigated and related to age and sex distribution laboratory and bacteriological findings The effect of rapid virological diagnosis on the clinical management of the patients has been evaluated, and attempts have been made to identify children at risk of contracting severe illness due to RSV infection

MATERIAL AND METHODS

Patients

A virological diagnosis of RSV infection was made on 432 patients admitted to the Paediatric Department Ulleval Hospital Oslo, from the autumn of 1972 until the spring of 1978 Retrospectively, clinical and laboratory data together with epidemiological and microbiological information were collected from the hospital records of 422 of these patients and transferred to punch cards Ten records could not be found The patients were grouped according to clinical diagnosis using the criteria given by Court (2), as seen in Table I

Microbiological studies

The virological methods used in this study are described in detail elsewhere (13) Briefly, nasopharyngeal secretions were collected from almost all patients and examined by indirect immunofluorescence for RSV antigens and inoculated into cell cultures for virus isolation In some cases the diagnosis of RSV infection was made by detecting a significant rise in antibody titre against RSV antigen in the complement fixation test

Nasopharyngeal and throat swabs were collected from most of the patients for routine bacteriological culture on

Table 1. *Distribution according to diagnosis and sex ratio in patients with RSV infection (422 patients)*

Diagnosis	No. of pts	%	Sex ratio M/F
Upper respiratory tract infections	33	7.8	0.4/1
Laryngitis	2	0.5	2 male
Bronchitis	14	3.3	0.4/1
Bronchiolitis	255	60.4	1.4/1
Pneumonia	98	23.2	2.5/1
Febrile convulsions	20	4.7	0.8/1
Otitis media as a complication to bronchiolitis and pneumonia	76	18.0	1.8/1

blood agar plates, chocolate agar plates and selective media for *Staphylococcus aureus*. Identification of *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Streptococcus pyogenes* in nasopharyngeal swabs was considered to be of potential pathogenic significance, whereas growth of *Staphylococcus aureus* and Gram negative rods was considered to represent colonisation in most cases. The culture findings from nasopharyngeal swabs are considered to be more reliable in reflecting the etiology of lower respiratory tract infection or otitis media than findings from throat swabs (6, 8), and only the former are included in this report.

Statistical analysis

Statistical analyses were performed using the Chi square test and Wilcoxon Mann Whitney rank test for two samples (W.M.W).

RESULTS

Diagnosis

Table 1 shows the distribution by diagnosis of the 422 patients. Most of the patients (86.9%) were admitted because of lower respiratory tract infections, of which bronchiolotis was dominating.

Patients with acute laryngitis are usually not admitted to the Paediatric Department in our hospital, and this probably accounts for two patients only in this group.

Otitis media occurred together with bronchiol... in 20% of these cases and in 13% of pa-ti... patients ...

Table 2. *Distribution according to age and diagnosis in patients with RSV infection (422 patients)*

Diagnosis	Age 0–6 mo (%)	6–12 mo (%)	1–2 y (%)	≥2 y (%)	No. of pats
Total RSV infections	34	27	18	21	422
Upper respiratory tract infections	21	15	18	46	33
Laryngitis	50	–	–	50	2
Bronchitis	14	14	14	58	14
Bronchiolitis	42	30	17	11	255
Pneumonia	24	27	17	32	98
Febrile convulsions	–	10	40	50	20
Otitis media as a complication to bronchiolitis and pneumonia	33	35	20	12	76

Age distribution

Whereas 72% of the children with bronchiolitis were below the age of one year, 51% of the children with pneumonia and 36% of the children with upper respiratory tract infection (URTI) only were found in this group (Table 2).

Sex distribution

The more severely affected children (bronchiolitis and pneumonia) showed male preponderance, whereas among the less ill patients (URTI) the opposite was found (Table 1) (Chi square = 15.45, $p<0.01$).

Bacteriological findings

Bacteriological studies by nasopharyngeal swabs were made on 342 patients (81%). The most common possibly pathogenic bacterium found among all the patients was *Streptococcus pneumoniae*, whereas among patients who had received antibiotics prior to the admission to hospital, *Haemophilus influenzae* was the most common. Only patients who had not received antibiotics prior to the admission to hospital, will be considered in the following evaluation of bacteriological findings in the different conditions.

Table 3 *Growth of potentially pathogenic bacteria from nasopharyngeal swabs in children with bronchiolitis only, bronchiolitis and otitis media, and pneumonia without otitis media*

Only patients without antibiotic treatment prior to admission are included in the table. The figures represent number of patients in each group. In some patients two bacteria species were found

Bacterial pathogen	Bronchiolitis without otitis	Bronchiolitis and otitis	Pneumonia without otitis
Streptococcus pneumoniae	25	15	11
Haemophilus influenzae	5	5	3
Streptococcus pyogenes	0	0	0
Totally positive	29 (27%)	17 (55%)	14 (42%)
Negative	29 (73%)	14 (45%)	19 (58%)
Total	107 (100%)	31 (100%)	33 (100%)

A comparison between the two major diagnostic groups, bronchiolitis and pneumonia, was considered to be of interest. As otitis media is often known to be a bacterial complication in RSV infections (4, 5), these patients were considered as a separate group. The following groups were compared: 1) Bronchiolitis without complications (107 patients), 2) Bronchiolitis complicated by otitis media (31 patients) and 3) Pneumonia not complicated by otitis media (33 patients). The number of patients with both pneumonia and otitis media was very small (14 patients) and therefore not included in the comparison.

Pathogenic bacteria were more often isolated from patients with bronchiolitis *and otitis media* than from patients with bronchiolitis only (Table 3) (Chi square = 7.12, $p<0.01$).

The difference between the positive bacteriological culture findings in patients with uncomplicated pneumonia and patients with uncomplicated bronchiolitis was not statistically significant (Chi square = 2.1, $0.2>p>0.1$).

Temperature on admission

Temperature on admission to hospital was compared among the same patient groups (Table 4). Patients with bronchiolitis and otitis (50 patients) had higher body temperatures than patients with uncomplicated bronchiolitis (205 patients) (W-M-W $p=0.017$).

Also patients with uncomplicated pneumonia (83 patients) had higher temperatures than patients with uncomplicated bronchiolitis (W-M-W $p=0.006$).

Leucocyte counts

Patients with bronchiolitis and otitis had higher leucocyte counts in peripheral blood than patients with bronchiolitis only (W-M-W $p=0.023$). Between patients with uncomplicated bronchiolitis and patients with uncomplicated pneumonia we found no significant difference in leucocyte count (W-M-W $p=0.21$) (Table 4).

Erythrocyte sedimentation rate

As regards ESR (Table 4) we found the difference between the 2 patient groups with *bronchiolitis not to* be statistically significant *on the 5%* level (W-M-W $p=0.06$). However, *the difference* in ESR between the patients with uncomplicated pneumonia and uncomplicated *bronchiolitis* was significant (W-M-W $p=0.003$). We found no significant difference in the distribution of positive and negative bacteriological findings when related to body temperature, leucocyte count and ESR among patients in the three patient groups (evaluated by the W-M-W test).

Antibiotic treatment, duration of stay in hospital and rapid virological diagnosis

Fig. 1 shows the median stay in hospital, the frequency of antibiotic treatment and the fre

Table 4. *Temperature on admission, leucocyte counts and erythrocyte sedimentation rate (ESR) in RSV infected children of different diagnostic groups*

The figures represent number of patients in each group

	Diagnosis		
	Bronchiolitis without otitis	Bronchiolitis and otitis	Pneumonia without otitis
Temperature, °C			
<37.5	57	5	16
37.5–38.0	50	13	20
38.1–39.0	62	22	16
>39.0	36	10	31
Total	205	50	83
Leucocytes × 10^9/l			
<5.0	22	1	8
5.0–10.0	103	24	40
10.1–15.0	56	15	26
15.1–20.0	6	7	6
>20	8	1	2
Total	195	48	82
ESR, mm/h			
<10	36	3	11
10–19	53	16	23
20–29	37	7	9
30–39	22	3	10
>40	12	8	19
Total	160	37	72

quency of rapid virological diagnosis by immunofluorescence among patients with uncomplicated bronchiolitis during the six winter outbreaks. The frequency of antibiotic treatment declined from 76–85% during the first three outbreaks to 35–41% during the last three outbreaks (Chi square = 36.8, $p<0.001$). Patients not diagnosed by rapid virological technique were more often treated with antibiotics than patients diagnosed by the rapid technique (Chi square = 9.5, $p<0.01$). The lowering of the median stay in hospital from 9 days in the first epidemics to 5–7 days in the later is significant (W-M-W $p<0.01$). However, we found no difference in median stay in hospital when related to rapid virological diagnosis for the individual patient (W-M-W $p=0.48$).

Mortality and symptomatic treatment

None of the children in this study died. Only one child was treated with intermittent positive airway pressure for a short period of time. All children with wheezing and respiratory stridor were placed in humidified oxygen tents, whereas symptomatic treatment by means of epinephrine, inhalation of vaporised racemic epinephrine and administration of other bronchiolytics were used according to symptoms. Of the 422 patients 18 patients received intravenous fluid therapy. Eight patients, critically ill, received steroid injections because of their severe bronchopulmonary obstruction.

Reinfections

Ten patients were admitted twice with verified

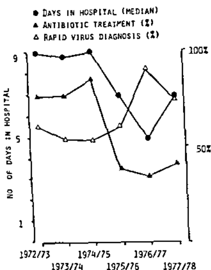


Fig 1 Duration of stay in hospital (median) frequency of antibiotic treatment and frequency of rapid virus diagnosis by immunofluorescence in RSV infected children with bronchiolitis without otitis media in each of the six out breaks

RSV infections The interval ranged from 7 months to 4½ years The first illness was usually more severe and the median stay in hospital was longer during the first illness (10 versus 8 days) The children with one exception suffered from bronchiolitis or pneumonia on both occasions

Frequency of obstructive airway disease

Among children older than two years at admission to the hospital 32% had previously suffered from bronchial asthma or obstructive bronchitis

Other disabling disease

Of the hospitalized children with RSV infections 13% had other disabling disease such as congenital heart disease cerebral palsy and leucemia These patients were distributed evenly throughout the different age groups

DISCUSSION

RSV infections occur frequently among the population during the winter season In adults and older children RSV usually causes trivial URTI but infants and small children are more susceptible to develop lower respiratory tract infection, and therefore more often need admission to the hospital Lower respiratory tract infection was the most common cause of admission to hospital in our patients (86 9%), and of these bronchiolitis was dominating Our finding that 61% of total admissions to hospital because of RSV infections and 72% of children with bronchiolitis were below the age of one year, reflects the severe illness caused by RSV in this age group and agrees well with the finding of others (14, 15)

The distinction between pneumonia and bronchiolitis has mainly been made on localised pulmonary consolidations as the clinical picture is often quite similar The children with pneumonia, however, were generally older than children with bronchiolitis, as Sims et al also found (15) The reason for this remains unknown, but perhaps older children require greater pulmonary changes to develop similar symptoms as the infants

More boys than girls had bronchiolitis and pneumonia (lower respiratory tract infections), as others have reported previously (14, 15) However, the less severely affected children (URTI) showed a distinct female preponderance We cannot explain this difference in sex ratio between the different diagnostic patient groups

Otitis media often complicates RSV infections In our study 18% of all patients had otitis media Gronroos et al considered RSV to be an important etiologic agent in otitis media (4) They isolated RSV from middle ear secretions, but they also found a high degree of bacterial superinfection in RSV infected children with otitis media, and the most common bacterium isolated from middle ear secretions was *Streptococcus pneumoniae* We found a higher frequency of positive bacterial findings from nasopharyngeal swabs in patients with bronchiolitis and otitis media than in patients with bronchiolitis only, and *Streptococcus pneumoniae* was the most common bacterium Patients with bronchiolitis and

otitis media had higher temperatures on admission, higher leucocyte counts and possibly a higher ESR ($p=0.06$) than patients with bronchiolitis only. Based on these findings, we believe that antibiotics should be used in children with RSV infections and signs of acute otitis media. It has been recommended to treat RSV infections with antibiotics in order to prevent otitis media (4). In our study otitis media occurred in only 18% of the RSV infected patients, and in almost all cases the signs of otitis were evident upon admission to the hospital. We will therefore not suggest prophylactic antibiotic treatment in RSV infections.

The pulmonary consolidations in patients with pneumonia may have been caused by RSV alone or by bacterial superinfection. It is possible that RSV may cause an immunological lung tissue reaction. A fourfold rise of specific antibody titre towards RSV was more common with increasing age in our patients. Our patients with pneumonia show a higher age distribution than the patients with bronchiolitis. The difference in bacteriological findings between patients with uncomplicated pneumonia and patients with uncomplicated bronchiolitis was not statistically significant. However, we did not perform more accurate studies of the bacteriology of the lungs of our patients, e.g. transtracheal aspiration or lung puncture, and the bacteriological examination was performed routineously without emphasis on scientific evaluation. The patients with pneumonia had higher temperatures at admission and higher ESR determinations, but we found no difference in leucocyte counts. Our findings do not support the hypothesis that the consolidations were caused by bacterial superinfection, neither do they prove the viral etiology in all cases. However, we do not believe that there ordinarily is indication for antibiotic treatment when pulmonary consolidations occur in the course of RSV caused bronchiolitis, but maintain that this must be assessed in each individual patient.

During the study period, a decreasing part of the patients with bronchiolitis was treated with antibiotics (Fig. 1) with no ill effects observed. This decrease was partly due to an increased efficiency of the rapid immunofluorescence technique. The median stay in hospital also fell during the course of the study, and this probably reflects earlier discharge from the hospital of patients with minor symptoms only. We believe that the change in attitude towards these patients was due to an increased awareness of the viral etiology in these cases among the clinicians, brought about by the virological surveillance.

Reinfections with RSV are common in childhood (5). The ten children in our study admitted twice with RSV infection, did not seem to be protected by their first infection when they contracted their second, although their second illness seemed less severe. Sims et al. also found that the children infected twice became less ill the second time (15).

RSV infections represent a great clinical problem in terms of affected children (13) and in terms of clinical illness in the individual patient. In our study there were no deaths due to RSV infections, and most authors refer low mortality figures, e.g. 0.5–1.3% (14, 15). However, the potentially serious prognosis of RSV infections is demonstrated through summons of 60 dead infants in Naples during the winter of 1978–79 caused by "il male oscuro", a disease entity most probably caused by RSV (1, 10).

The greater susceptibility of asthmatic children to react with bronchial obstruction in the course of virus infections is well documented (7, 12). This is demonstrated by the frequency of previously diagnosed asthma in RSV infected children older than 2 years in our study (32%). Several authors draw attention to the link between bronchiolitis in infancy and the subsequent development of bronchial asthma (3, 11). Long term effects on the lower airways may result from an initial attack of bronchiolitis (9). Thus RSV infections may be important and have long lasting effects beyond the initial clinical illness.

ACKNOWLEDGEMENT

We thank Dr J Eng in charge of the bacteriological section of the *Microbiological Laboratory, Ullevaal Hospital*, for helpful discussions and I Holme Head of The Life Insurance Companies Institute for Medical Statistics, Ullevaal Hospital, for statistical advice Financial support was received from Oslo kommunes medisinsk vitenskapelige stipendier 1979

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Submitted Febr 27, 1980
Accepted May 22, 1980

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Acta Paediatr Scand 69 731–734, 1980

SUCCESSFUL MANAGEMENT OF BACTERIAL ENDOCARDITIS OF THE MITRAL VALVE DUE TO *STAPHYLOCOCCUS EPIDERMIDIS* IN AN IMMUNOSUPPRESSED HOST

JAQUES BELIK, GEORGE FLINN, GASTON RIVERA and SHYAMAL K SANYAL

From the Cardiopulmonary Disease

ABSTRACT Belik, J, Flinn, G, Rivera, G. and Sanyal, S. K. (Cardiopulmonary Disease Service, Hematology Service and General Pediatrics Division, St Jude Children's Research Hospital, Memphis, Tennessee, USA). Successful management of bacterial endocarditis of the mitral valve due to *Staphylococcus epidermidis* in an immunosuppressed host. Acta Paediatr Scand, 69: 731, 1980. — We report the successful management of bacterial endocarditis ... *Staphylococcus epidermidis*, in a 34-month-old boy with a ... mode ... the evo...

KEY WORDS Bacterial endocarditis, immunosuppressed host, *Staphylococcus epidermidis*, echocardiography

The propensity of immunosuppressed patients to develop systemic infection is well recognized Despite increased susceptibility to infection, these patients rarely develop bacterial endocarditis (1, 2), in a 21-year study of 7840 patients with cancer, only 28 (0 35%) developed infective endocarditis (2) In recent years echocardiography has come into wide use as a non-invasive means of detecting valvular vegetations—the hallmark of endocarditis (3–6) Our purpose is (i) to report the successful management of endocarditis due to a penicillin resistant strain of *Staphylococcus epidermidis* in a child with acute lymphocytic leukemia (ALL) who had been receiving immunosuppressive chemotherapy, and (ii) to describe the serial echocardiographic findings during the course of his disease

CASE REPORT

A 34-month-old boy with ALL was admitted with fever, ... days' duration Physical findings showed a pale child with an elevated temperature (39 7°C), heart rate (142/min), and respiratory rate (32/min) Blood pressure was 112/76 mmHg He had oral ulceration, hepatomegaly and generalized petechiae There was no heart murmur Hemoglobin was 4 5 mmol/l white blood cell count, 1 1×10⁹/l with blast cells and no neutrophils The platelet count was 8 0×10⁹/l Urine analysis and a chest roentgenogram were normal Cultures of urine, blood, throat and cerebrospinal fluid were sterile

Fever and neutropenia suggested septicemia Alternate induction chemotherapy for inherently drug resistant ALL was stopped, blood was obtained for further culture and antibiotic therapy was started first with intravenous oxacillin (3 g/sq m per day) and gentamicin (150 mg/sq m per day) and 8 days later, with chloramphenicol (100 mg/kg per day) and clindamycin (1 5 g/sq m per day) However, there was little improvement

On the 12th day of hospitalization the patient developed a fine macular rash persistent tachycardia, and an ejection systolic murmur The spleen was palpable at 4 cm below the left costal margin A presumptive diagnosis of bacterial endocarditis was made and an echocardiogram was obtained (4, 5) with a 3 5 mHz transducer The right and left ventricular cavity dimensions the shortening

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Supported in part by USPHS Grant CA21765-02 from the National Cancer Institute, and by ALSAC

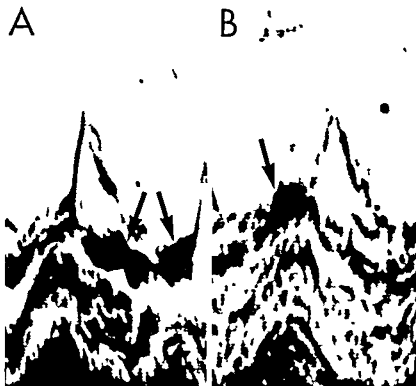


Fig 1 Serial changes in echocardiographic features during the course of disease in our patient The initial study (panel *a*) shows the presence of an irregular echo-producing mass (black arrows) that was attached to the anterior and posterior leaflets of the mitral valve Note the striking decrease in the echo mass (black arrow panel *b*) shortly after the patient had a generalized seizure

fraction mean velocity of circumferential fiber shortening and left atrial/aortic root ratio all were normal The mitral valve recording showed an echo producing mass that was attached to the anterior and posterior leaflets (Fig 1*a*) The motion of the valve leaflets however was unrestricted No abnormal echoes were seen in the left atrium or the left ventricular outflow tract

Multiple blood cultures grew (7) *S epidermidis* that was sensitive to cephalothin and gentamicin these drugs were given intravenously at 1 2 g/m² and 240 mg/m² respectively each day for 4 days Later the therapy was changed to vancomycin (30 mg/kg per day) on the basis of minimal inhibitory concentration and minimal bacterial concentration studies

Forty eight hours after the diagnosis of bacterial endocarditis the patient had a generalized seizure that lasted for 1 min Spinal fluid was normal as were the serum levels of glucose and electrolytes including calcium and magnesium Echocardiogram showed a decrease in the size of the previous echo mass (Fig 1*b*) A computed axial tomogram (CT scan) of the skull showed a lenticular shaped lucency in the left posterior parietal region Phenobarbital and phenytoin were given

The patient also developed congestive heart failure and hypoxemia that responded well to digitalis diuretics and oxygen therapy The patient continued to be febrile and on the 25th day a blood culture grew *Klebsiella pneumoniae* Amikacin was added to vancomycin therapy and the patient became afebrile within 48 hours Echocardiogram (Fig 2) and CT scan of the skull were normal The patient was discharged from the hospital Follow up examination 3 months later showed no heart murmur or neurologic dysfunction Serial echocardiograms remained normal

COMMENTS

The epidemiology of infective endocarditis has shown marked changes in recent years including the emergence of *S epidermidis* as a major cause of endocarditis especially among



Fig 2 Echocardiographic features during convalescence After the patient became afebrile follow up studies showed complete disappearance of the echo mass

those who have had open-heart surgery or pre-existing heart disease (8, 9) However, involvement of a previously normal heart, as noted in our patient, is quite rare Although unusual, the present case again emphasizes the importance of an early diagnosis of endocarditis and prompt institution of effective antibiotics, selected on the basis of results of sensitivity studies *in vitro*

An early diagnosis of endocarditis in a child with ALL presents certain problems since the clinical hallmarks of bacterial endocarditis (such as pyrexia, petechiae, and palpable spleen) are also common findings for children with ALL in hematologic relapse Likewise, in the presence of anemia, heart murmur and tachycardia are of little diagnostic value Multiple positive blood cultures indicated septicemia in our patient Echocardiography showed an irregular echo mass attached to the anterior and posterior leaflets of the mitral valve The non uniform thickening of the valve the lack of restricted motion of the valve leaflets, and the attachment of the mass to these valve leaflets, without extension into the left atrium suggested the diagnosis of valvular vegetations, the hallmark of endocarditis (3, 5, 10) A striking reduction in the echo mass after a generalized convulsion strongly suggested an embolism in the central nervous system This temporal sequence of events in our patient—an embolic episode followed by an apparent decrease in the mass of valvular vegetation—is similar to that reported by Roy and co-workers (11) and others (12, 13)

The sensitivity of echocardiography for detecting valvular vegetation has evoked considerable debate Wann et al (14) could detect echocardiographic evidence in 34 percent of their patients who had bacterial endocarditis and concluded that echocardiography does not provide a sensitive means for detecting valvular vegetations By contrast, Andy and co-workers (15) identified echocardiographic abnormalities suggestive of endocarditis in 11 of 12 patients who had clinical evidence of mitral valve involvement Our case further documents that echocardiography does provide a safe, non invasive means for detecting valvular vegetations in children suspected of having bacterial endocarditis (4, 5) and that serial echocardiography provides important information about the evolution of the disease in individual patients.

Finally, it has been suggested that individuals with infective endocarditis who develop vegetations that are large enough to be detected by echocardiography have a poor prognosis and frequently require valve replacement (6, 14) Our observations indicate that echocardiographic documentation of valvular vegetation per se does not invariably signify a poor prognosis

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Submitted Jan 7, 1980
Accepted April 21 1980

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Acta Pædiatr Scand 69 735–739, 1980

BORDERLINE GALACTOSEMIA

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ABSTRACT Pettersson, R , Dahlqvist, A , Hattevig, G and Kjellman, B (Department of Paediatrics Central Hospital, Skövde and Department of Nutrition, University of Lund, Sweden) Borderline galactosemia Acta Paediatr Scand, 69 735, 1980.—A family with combined heterozygosity for "classical" galactosemia (deficiency of uridyl transferase) and for galactokinase deficiency is reported The proband, who had this genetic combination was detected as newborn in the ordinary screening for galactosemia A lactose tolerance test at the age of three months proved normal and he has no symptoms or signs on ordinary diet The mother of the proband was not only heterozygot for "classical" galactosemia and galactokinase deficiency but also for the Duarte variant She had a substantial urine excretion of galactose and high serum galactose after an oral lactose load She had no clinical symptoms or signs Patients with combined heterozygosity for galactosemia may develop cataracts and should be followed by clinical examinations

KEY WORDS Galactosemia, Duarte variant, galactokinase deficiency

The basic enzyme defect in 'classical' galactosemia is an almost total deficiency of the *uridayl transferase activity* (Fig 1) The frequency of homozygosity is 1–4 per 100 000 births (14)

A number of *variants* of galactosemia due to different kinds of defects of the uridyl transferase activity have been described (Table 1)

Furthermore, deficiencies of *galactokinase* and of *epimerase* exist (Fig 1)

Deficiency of galactokinase implies insufficient phosphorylation of galactose, thus galactose and galactitol accumulate in tissue and body fluids and this may cause cataracts The incidence of this disorder ist estimated to one per 50 000 (15)

Deficiency of epimerase is estimated to occur in about one per 40 000 (10) No certain clinical disorder is related to this deficiency

Disturbances of the galactose metabolism can thus be complex and combined deficiencies e g combined heterozygosity may cause unclear clinical symptoms and laboratory signs (12) The present study illustrates such problems

METHODS

Screening of newborns for galactosemia is done with the microbiological method of Guthrie (11)

Galactose 1 phosphate was measured according to the fluorimetric method of Dahlqvist (8) The activity of *uridyl transferase* in erythrocytes was estimated by UDPG consumption test (8) at the Department of Nutrition Lund Electrophoretic characterization of the mobility of uridyl transferase was done according to Beutler & Mathai (3) and performed at the Metabolic Laboratory of Rigshospitalet, Copenhagen

The activity of *galactokinase* in erythrocytes was measured with the method of Mayes & Guthrie (15), which is a modification of the method originally described by Sherman (19)

The activity of *epimerase* in erythrocytes was assayed as described by Gitzelman et al (9) This assay, as well as that of galactokinase were performed at the Department of Nutrition Lund

Lactose tolerance test was done with 2 g lactose per kg body weight Maximal dose was 100 g

Serum galactose was measured every 15 min and urine galactose every hour after the load, using the method mentioned above

RESULTS

Case histories

The proband (M A 78 01 27, male) was born after a normal pregnancy and normal delivery Birth weight was 3 520 g and the neonatal

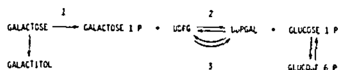


Fig 1 Schematic pathway of galactose metabolism (1) = galactokinase, (2) = galactose 1 phosphate uridyltrans ferase, (3) = uridinediphosphate galactose-4 epimerase

period was uneventful Routine screening for galactosemia on the fifth day after birth was positive (0 50 mmol galactose/l blood), this was confirmed in a screening a few days later (1 9 mmol galactose/l blood) Galactose-1-phosphate in RBC was 0 018 mmol/l at the age of one month An assay of galactose-1 phosphate in blood from the screening specimens was performed about two years later and showed values below the upper normal limit of newborns The boy was given a lactose-free diet during the first three months After that normal feeding was started We have now followed the boy for two years He has developed normally and repeated thorough clinical examinations, including ophthalmoscopy have not disclosed any abnormalities Liver function tests were normal

The initial laboratory findings of the proband led us to perform an extensive study of his whole family, i e his mother, father and sister Furthermore, five siblings of the mother, and her mother, joined the study The father of the mother was dead The only member of the family with symptoms which might have connection to galactosemia was the mother

The mother of the proband (J A 49 11 06) had a normal neonatal period but from the age of 4 months she was hospitalized for 6 months due to failure to gain weight and serious respiratory distress leading to tracheotomia The exact nature of the respiratory symptoms could not be identified During the hospital stay repeat routine tests for reducing substances in urine were negative During the following year she suffered recurrent admissions to hospital because of respiratory distress After that she gradually recovered from her symptoms At the time of this study she is asymptomatic except for a mild seasonal rhinitis due to an allergy to birch pollen A thorough clinical examination, including an examination by an ophthalmologist proved normal Liver function tests were normal

Analysis of uridyl transferase

This analysis was performed on all members of the family The mother had values as low as those we have earlier measured in patients with 'classical' galactosemia Intermediate values corresponding to heterozygotes for 'classical' galactosemia were found in the proband his sister two of the siblings of his

Table 1 *Variants of galactosemia due to different deficiencies of the uridyl transferase activity*

	Red blood cell		
	Transferase activity (% of normal)	Transferase mobility	Transferase stability
Classical galactosemia	0	–	–
Symptomatic transf var			
(*a*) Rennes variant (18)	7	slow	not tested
(*b*) Indiana variants (6)	40	–	unstab (4°C)
(*c*) Negro variants (1)	0	–	–
Asymptomatic transf var			
(*a*) Duarte variants (2 3)	50	fast	stable
(*b*) Los Angeles variants (16)	100+	fast	not tested
(*c*) Chicago variants 1 (7)	100	normal	unstab (4°C)
Chicago variants 2 (7)	25	fast	stable

Table 2 *Results of enzyme analyses in a family with combined heterozygosity for galactosemia*
- The porband s grandmother his sister and 2 of 5 siblings of his mother had low uridyl transferase (10 1–14 5 IU/gHb)

	Spot test	Uridyl transferase IU/gHb	Uridyl transferase Mobility	Galactokinase (µmol/hour/ml erythrocytes)	Epimerase (µmol/hour/ml erythrocytes)	Lactose tolerance test
Proband	Neg	12 5[a]	Norm	0 33[b] 0 18[b]	6 6	Norm
Mother	Pos	4 0[c]	Fast	0 13	–	Abnorm
Father	Neg	29 5	Norm	0 19	–	–
Controls adults		26 5 (*n*=20)		0 22 (*n*=12)	4 9 (*n* = 36)	
S D		4 5		0 03	1 13	

ctokinase in newborns is about three times that

mother and in his maternal grandmother The father of the proband and the remaining three siblings of the mother had normal values (Table 2)

Electrophoresis of the uridyl transferase for studying the mobility of the enzyme was performed on specimens of the proband his mother and his father The enzyme mobility was normal for the proband and his father but fast for his mother indicating that she was heterozygot for the Duarte variant (Table 2)

Analysis of galactokinase

This analysis was performed on the proband and his parents The proband had a value about half of that for a normal three weeks infant and the mother an activity half of that seen in normal adults The father had an enzyme activity within the range of the controls (Table 2)

Analysis of epimerase

This analysis was performed on the proband and proved normal (Table 2)

Lactose tolerance test

This test was performed on the mother and on the proband at the age of 3 months The results of the proband proved normal His mother had a maximal serum galactose of 2 4 mmol/l after two hours Her maximal urine galactose was 16 5 g/l Six hours after the load her red blood cells contained 0 06 mmol/l of galactose-1-phosphate, which is about one third of the values we have found in patients with "classical galactosemia on diet

DISCUSSION

The biochemical findings in the proband can only be explained by a double heterozygosity, i e he is heterozygot for both "classical galactosemia and galactokinase deficiency (Fig 2) Evidently this genetic compound can give chemical signs detectable in a routine screening during the newborn period Heterozygotes for galactokinase deficiency seem to be clinically normal, but in a recent study a cause and effect relation of such genetic state to presenile cataract is suspected (17) One may speculate whether there is an increased risk of future symptoms in our proband with his double heterozygosity We have found but one previously reported case with symptoms due to such a genetic compound (17)

Our proband does not have any symptoms or signs, he develops normally and liver function tests and lactose tolerance are normal We therefore intend to continue his normal diet and follow him with yearly examinations

The biochemical pattern found in the mother of the proband implies that she is three fold heterozygote, i e for the Duarte

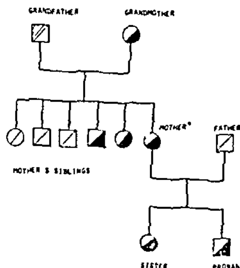


Fig 2 Pedigree of family with combined deficiency of uridyl transferase and galactokinase The grandfather is dead and his genotype is estimated * indicates heterozygosity for galactokinase ◪ ◑ Heterozygosity for classical galactosemia ⊘ ⊘ Heterozygosity for Duarte variant

variant, "classical" galactosemia and galactokinase deficiency (Fig 2) We have not been able to find any previous report of such a genetic state

Combined heterozygosity for the Duarte variant and classical galactosemia has been reported previously and seems to give no symptoms on ordinary diet (13), but lactose tolerance tests can be cleraly abnormal (5)

As the mother had a substantial excretion of galactose in urine and high serum galactose after the lactose load she was considered for dietary therapy She has, however no symptoms and signs on ordinary diet and had normal liver function tests Furthermore we are not able to relate the severe symptoms during her infancy to her genetic combination We therefore have recommended her an ordinary diet but not more than one glass of milk daily

Results of our study actualize once more the question where the critical limit should be placed in screening of newborns for galactosemia (12, 13, 20) In Sweden this limit is now 0 5 mmol galactose/l serum

In an analysis of the results from the Swedish screening programme 12 true cases of galactosemia and 137 "false" cases were found during the period 1967–1977 (20) There were some "false" cases with an initial serum galactose concentration above 5 mmol/l These 137 cases are "false" in the sense that they are not homozygotes, but it is possible that they include combined heterozygotes Is it worth the trouble and the costs to make extensive enzyme analyses in these false cases in order to substantiate some with a combined genetic state? If these children are at a greater risk for e g development of cataract, we think such analyses are worth-while However, such a risk is so far only suspected and not proven (17)

ACKNOWLEDGEMENT

We acknowledge help from Niels J Brandt M D and Ernst Christensen Ph D Metabolic Laboratory Rigshospitalet Copenhagen

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Submitted March 5 1980
Accepted May 13 1980

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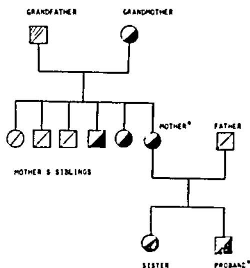


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Acta Pædiatr Scand 69 741–745, 1980

CHANGES IN PERIPHERAL BLOOD CELLS AND SERUM FERRITIN IN LYSINURIC PROTEIN INTOLERANCE

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ABSTRACT [illegible] Hospital, [illegible] serum ferritin in lysinuric protein intolerance [illegible] autosomal recessive disorder Twenty seven patients with lysinuric protein intolerance, an of diamino acid transport, have been followed at our hospital since 1965. Many of the pa- tients had recurrent mild normochromic anaemia without apparent iron deficiency, and leuko- and/or thrombocytopenia Serum ferritin concentrations were inappropriately high. Serum ferritin concentration appears to be a valuable index of the control of this disease.

KEY WORDS. Blood cells, ferritin in serum, amino acid metabolism, inborn errors, lysinuric protein intolerance

Lysinuric protein intolerance (LPI) (McKusick 22270), first described in 1965 (13), is a disorder marked by periodic hyperammonaemia and increased urinary excretion of the diamino acids lysine, arginine and ornithine The disease is inherited as an autosomal recessive trait (12) A defect in diamino acid transport is present in the kidney tubuli (22) and jejunal epithelium (15), and probably in the liver cells (20, 21) The liver cells evidently are deficient in the necessary urea cycle intermediates arginine and ornithine, and urea synthesis is impaired This results in hyperammonaemia after protein intake, and in protein aversion and malnutrition As the renal transport defect is severest for lysine, patients are inevitably deficient in this essential amino acid also The clinical signs of LPI include hepatomegaly and often splenomegaly (23)

Hyperammonaemia, lysine deficiency, hepatomegaly and splenomegaly may all predispose to haematological disturbances (7, 8) In fact, patients with LPI often have mild normochromic anaemia and leuko- and thrombocytopenia (1, 3, 5, 11, 13, 23). Among the amino acid transport diseases these findings may be specific for LPI, since they are not present in (protein-tolerant) patients with hyperdiaminoaciduria (9, 25) or cystinuria (17)

The need of this closer analysis of haematological changes in LPI was prompted by the death of one of our 27 patients after a short episode of haemolytic anaemia, thrombocytopenia and massive ferritinaemia. Haematological abnormalities are frequent enough to be regarded as useful minor criteria for the diagnosis of LPI. Moreover, serum ferritin measurements appear useful for assessment of the control of the disease

PATIENTS

Twenty seven patients with LPI were seen at our hospital a total of 261 times from 1965 to 1978 Twenty of them have been described earlier (23) During the last 2 years all patients were seen at 6-month intervals This study examines the laboratory data at the first and each subsequent visit and first set of data at each admission The treatment consisted of a low protein diet, 1–1 5 g/kg daily with a supplement of arginine-HCl, or during the last 2 years, of citrulline, alone or with lysine-HCl Citrulline is effective in preventing hyperammonaemia and orotic aciduria in the patients (14)

Most patients had been without major haematological problems Five of them, however, had anaemia requiring blood transfusions on one or more occasions Two such histories are given

Patient 1 (StK), a girl with intrauterine rubella syndrome, was diagnosed as having LPI at 2 years of age During her third and fourth years of life she presented

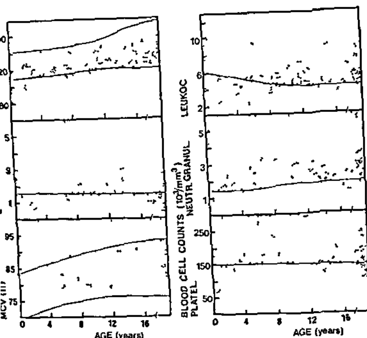


Fig 2 Peripheral blood RBC WBC and platelet counts versus age in 27 patients with LPI Shading indicates the normal ranges (6 26)

concentrations of other proteins produced in the liver were normal or increased (10) Two patients had a slightly decreased concentration of factor VII in absence of bleeding or illness For the other clotting factors tested in 5 patients levels were normal

In bone marrow aspirates ($n=10$) the distribution and morphology of the blood cell precursors were normal

Serum iron concentration was always within normal limits (range 7 1–29 5 mean 16 7 μmol/l in 99 samples from 27 patients normal 7–31 μmol/l) The total iron binding capacity varied from sub- to supranormal values (19 7–91 6 mean 59 7 μmol/l normal 45–70 μmol/l)

In 95/99 measurements transferrin iron saturation was more than 15% Although iron deficiency seemed unlikely in the patients the osmotic fragility curve of the erythrocytes often showed peculiarly increased resistance This was most marked before incubation for 24 h (Fig 3) Such an increased resistance is usually seen in either iron deficiency or liver disease In these however it is further increased by incubation of the cells for 24 h (7)

Serum ferritin concentration was strikingly high and nearly always far above the upper limit of the normal range (Fig 4) The individual values showed no correlation with the abnormalities in peripheral blood cells, or with serum levels of iron TIBC, ASAT, ALAT or LDH, or with the presence of hyperammonaemia as reflected by orotic aciduria Ferritin concentrations were highest during stress situations , as shown by the following examples (see also Patients 1 and 2 in Patients)

The ferritin levels rose in one patient during

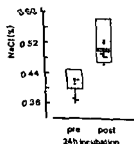


Fig 3 Osmotic fragiligy of erythrocytes in patients with LPI The values are sodium chloride concentrations resulting in 50% haemolysis before and after incubation for 24 h The dots represent the values for the patients and the shaded areas the reference ranges

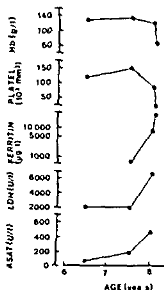


Fig 1 Course of blood haemoglobin concentration and platelet count serum ferritin concentration and activities of lactic dehydrogenase and aspartateaminotransferase in a girl with LPI ending at her death at the age of 8 years

with four episodes of respiratory or urinary infection associated with uncompensated haemolytic anaemia (Hb 69–78 g/l) maximal reticulocyte count (3 4%) and thrombocytopenia (20–30 000/mm³) During each episode she required one or more blood transfusions The anaemia was associated with increases in serum ferritin (highest recorded level 2700 µg/l normal 7–140 µg/l) lactate dehydrogenase (LDH 1600 U/l normal <620 U/l) and ASAT (87 U/l normal <40 U/l) Patient 2 (SK) a girl her LPI diagnosed 2 years earlier died at the age of 8 years after 1 month with a febrile pulmonary disease vomiting and diarrhoea proteinuria anaemia and thrombocytopenia Her Hb was 75 g/l platelets below 15 000/mm³ and reticulocyte count below 0 6% She had large increase in serum ferritin LDH and ASAT (Fig 1)

METHODS

Haemoglobin red blood cell indices and leukocyte and platelet counts were determined with a Coulter counter Reticulocytes were counted microscopically Serum iron and total iron binding capacity were measured spectrophotometrically (4 16) and serum ferritin by a radioimmunoassay (19) Erythrocyte osmotic fragility was determined before and after incubation for 24 h (7) Haptoglobin was determined as haemoglobin binding capacity (24)

RESULTS

None of the patients had evidence of major bleeding although they often had bruises in the lower limbs, as a sign of capillary fragility

The neonatal histories revealed petechiae in 6 patients

The patients had 5–16 (average 10) haematological work ups during the past 13 years In every patient one of the following abnormalities had been found at least once anaemia (in 16 of the 27 patients), peripheral leukopenia (23/27) or thrombocytopenia (24/27) (Fig 2) The leukopenia was due to a decreased number of neutrophil granulocytes However the findings were not constant in any of the patients the patients with anaemia had subnormal concentrations of Hb on 38 of 166 occasions, the patients with leukopenia had subnormal leukocyte counts on 69/219, and the patients with thrombocytopenia had subnormal thrombocyte counts on 82/224 occasions Every patient occasionally had evidence of abnormal red cell morphology in the form of poikilocytosis (86/162) and/or anisocytosis (132/162) Macrocytosis and spherocytosis were less common Blood folate and serum vitamin B_{12} concentrations were normal The tendency to macrocytosis (Fig 2) could thus not be due to a deficiency of these vitamins Further no signs of defects in haemoglobin synthesis or red cell metabolism were found, erythrocyte protoporphyrins urinary and faecal porphyrin intermediates, Hb electrophoresis and erythrocyte 2 3 diphosphoglycerate and serum fetal Hb concentrations were normal Some of the changes in the peripheral blood cells were age dependent (Fig 2) Anaemia was common only until the age of 3 years Blood cell pathology did not correlate with the prevalence of hyperammonaemia as reflected by orotic aciduria The anaemia was associated with haemolysis the reticulocyte count was increased in 26/27 patients (total range from 0 4 to 9 2% in 168 samples) and in 96 determinations it was more than 1 5% Haptoglobin data indicated that the haemolysis was intravascular The haemoglobin binding capacity ranged from 0 01 to 0 14 g/l (mean 0 04 normal in our laboratory >0 3 g/l) The subnormal values were unlikely to be due to decreased hepatic synthesis, as

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Submitted March 3 1980
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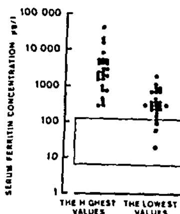


Fig 4 The highest and lowest serum ferritin levels in patients with LPI during a follow up period of 2 years The interval between determinations was at least 6 months The rectangle indicates the normal range (mean ± 2 S D) (19)

varicella from 295 to 15200 µg/l, in another during a febrile vaginal herpes infection from 425 to 20500 µg/l, and in a third with hyperammonaemic coma from 318 to 1848 µg/l The concentration rose from 380 before pregnancy to 1650 µg/l by the 9th week in one patient and another had 8200 µg/l at the time of delivery

DISCUSSION

In LPI, the presence of blood cell abnormalities may be helpful as a minor criterion in the diagnosis, especially in the youngest infants who may fail to show hyperammonaemia after the diagnostic i v alanine load (23) Similar changes have been reported in the blood of some patients with primary deficiencies in urea cycle enzymes (18) The hyperammonaemia itself, however, can hardly be the cause of the abnormalities, as the severity of the changes did not correlate with the magnitude of the orotic aciduria Also, the prevention of hyperammonaemia with a citrulline supplemented low protein diet did not influence the blood cell changes A more likely reason is amino acid imbalance and deficiency Lysine deficiency may be a single important cause Rats and piglets fed on lysine deficient diets have shown haematological changes resembling those seen in LPI intravascular haemolysis and a decreased capacity of the bone marrow to respond with cell production (2, 8) We thus postulate that in LPI these features may be signs of lysine deficiency

The concentration of serum ferritin in healthy subjects is believed to reflect the size of the iron stores It also correlates with the amount of stored iron when the level is increased, for instance in liver affections and in inflammatory and neoplastic diseases (27) However, in patients with LPI this correlation does not exist No stainable iron has been found in the bone marrow of the patients with LPI, and accumulated ferritin iron was not found in liver samples (Rajantie et al, unpublished data)

LPI should be remembered as a possible cause of high values of serum ferritin in children Serum ferritin appears to be an indicator of the control of the metabolic disorder in patients with LPI and should thus be measured frequently As was seen in patient 2 unexpected rises may predict exacerbations of the disease

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Acta Pædiatr Scand 69 747–751, 1980

SERUM LIPIDS IN MATERNAL/CORD BLOOD PAIRS FROM NORMAL AND LOW BIRTHWEIGHT INFANTS IN DAR ES SALAAM, TANZANIA

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ABSTRACT Boersma, E R (Department of Child Health, Muhimbili Medical Centre, incorporating the Faculty of Medicine, University of Dar es Salaam, Tanzania) Serum lipids

appropriate for gestational age and 17 small for dates infants and their mothers immediately after delivery in Dar es Salaam, Tanzania The mean serum level of total cholesterol in cord blood of term appropriate for gestational age infants was 1 9 mmol/l, whereas that from their mothers 6 3 mmol/l Cord serum triglyceride values in term appropriate for gestational age infants were 0 29 mmol/l and from their mothers 1 91 mmol/l Results of these maternal cord serum lipids in the normal and low birthweight groups are similar to those of Western countries

KEY WORDS Serum lipids, maternal/cord blood, normal term infants, low birthweight infants Tanzania

The metabolism of lipids seems to be important to the development of the embryo and fetus In man total body fat increases from about 1 to 12–16% by weight between the 26th and 40th week of gestation (21 27) Prenatally, lipid synthesis predominates over lipid breakdown After birth the stored fat serves as an important source of energy when the limited glycogen stores tend to become exhausted The most rapid change in serum lipids occurs in the first year of life For example in Western countries between birth and 1 year of age total cholesterol has increased by 130 to 240% (6 11) and triglycerides by 68% (11)

The incidence of arteriosclerosis in Tanzania is low Cholesterol values in adulthood have also been found to be low in other developing countries (4 20 23 25) which would be regarded by many to be a factor in the low incidence of arteriosclerosis, the precursor of coronary heart disease (26) In order to gain further understanding of the role of serum lipids during intrauterine life in a community living in completely different socio economic and nutritional conditions from those in Western countries the serum total cholesterol and triglycerides levels were measured in Tanzanian mothers and their infants immediately after delivery These maternal/cord serum lipids for preterm appropriate for gestational age, small for-dates and term appropriate for gestational age infants were compared with data from similar studies from Western countries

MATERIALS

This survey forms part of a study of the interaction between malaria and pregnancy carried out between September 1976 and September 1977 at the University Hospital Muhimbili Medical Centre and the Government Maternity Ocean Road Hospital For details regarding local health facilities population geographical and socio-economic circumstances and local intrauterine growth standards see previous studies (3 19)

Maternal venous blood and cord blood was obtained from 300 fasting mothers and their offsprings immediately after delivery From this population the following groups

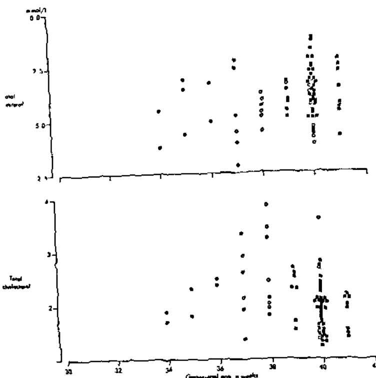


Fig 1 Individual values for serum total cholesterol in mothers (upper part) of preterm AGA (●) small for-dates (○) and term AGA (■) and those of their offspring plotted against gestational age (lower part)

blood Immediately after birth 5 ml of venous blood was

analysed

METHODS

dam The Netherlands

The correlation coefficient (r) of the investigation performed in Dar es Salaam and those in Rotterdam was 0 9 or greater Where applicable statistically significant differences between means were measured by Student s t test a p value $<0\ 05$ was considered as significant

RESULTS

Table 2 shows the fetal and maternal concentrations of T C and TG of the analysed sera in the 3 groups immediately after delivery T C and TG showed a virtually normal distribution in the three different groups of maternal/cord serum pairs, except for TG in cord serum of the three different groups of infants, where a skewed distribution toward high concentrations was found

Maternal serum lipids Mean T-C level in maternal sera in mothers of term AGA infants was 6 3 mmol/l (range 4 1–9 2 mmol/l) Mean maternal T C level in mothers of term AGA infants was significantly higher than that found in mothers of preterm AGA and SFD infants ($p<0\ 05$ and $p<0\ 01$ respectively) Inspection of the individual values of T-C in the 3 groups studied, as plotted against gestational age in Fig 1 (upper part), suggests a slight increase from 34 to 40 weeks' gestation Mean TG values were higher in the 2 groups of mothers giving birth to AGA infants (preterm or term), compared to the mothers of SFD infants ($p<0\ 01$)

Cord serum lipids In the corresponding cord sera of term AGA infants mean T C value was 1 9 mmol/l (range 1 2–2 8 mmol/l) Mean cord level of term AGA infants was quite similar to that of preterm AGA, but slightly lower

Table 1 *Anthropometric and clinical data (mean ± S D) of 54 term AGA, 14 preterm AGA and 17 small for-dates infants and their mothers*

Number of determinations in parenthesis

Data	Term AGA	Preterm AGA	Small for-dates
Mothers			
Age years	24 2±4 5 (52)	21 7±5 0 (13)	21 4±4 6 (17)
Parity	3 5±1 9 (51)	2 0±2 0 (14)	2 6±2 1 (17)
Height cm	153 5±7 1 (50)	152 7±3 1 (14)	152 6±4 7 (17)
Arm circumference cm	25 0±2 6 (51)	24 8±2 0 (14)	24 4±1 9 (17)
Infants			
Ratio males/females	26/28	7/7	8/9
Birthweight g	3 036±262 (54)	2 096±464 (13)	1 610±470 (17)
Length cm	48 5±1 7 (54)	44 5±2 9 (13)	43 4±3 6 (17)
Head circumference cm	34 4±1 0 (54)	31 4±2 2 (13)	30 5±1 5 (17)

of newborns and their mothers were studied in more details

Group 1 54 mothers and their term appropriate for gestational age infants (controls) A term appropriate for gestational age infant (term AGA) is defined as an infant who is born within 38–42 weeks of gestation and whose birthweight is within ±1 S D from the mean for that particular gestational age using the local standards (3) The gestational age of these infants ranged from 38 to 41 weeks

Group 2 14 mothers and their preterm appropriate for gestational age infants The gestational age of these infants ranged from 34 to 37 weeks A preterm appropriate for gestational age infant (preterm AGA) is defined as an infant who is born before 37 completed weeks of gestation and whose birthweight is within the mean±1 S D for the gestational age using the local standards (3)

Group 3 17 mothers and their small for dates infants with a gestation ranging from 37 to 40 weeks Small for dates (SFD) are defined as infants whose birthweight is below the 10th percentile value for gestational age according to the same standards (3)

Mothers excluded were those with hypertension with or without proteinuria and conditions complicating pregnancy such as diabetes multiple pregnancies and antepartum hemorrhage or fetal malformations All infants included were born by normal vaginal delivery and their one minute Apgar score was 9 or 10 Standing height of mothers was recorded in centimeters The left midupper arm circumference was taken by a non stretchable tape measure to the nearest 0 5 cm Anthropometric measurements were taken from the newborn infant immediately after delivery Gestational age of the neonate was assessed of

data of the 3 different groups of mothers and their infants are summarised

At delivery the umbilical cord was carefully cleaned before collection of approximately 10 ml of mixed cord

Table 2 *Serum concentration of total cholesterol and triglyceride in maternal and cord blood of the three different subgroups Mean ± S D*

Total number of determinations in parenthesis

	Mothers			Babies		
	Term AGA	Preterm AGA	SFD	Term AGA	Preterm AGA	SFD
Gestation range in weeks	38–41	34–37	37–40	38–41	34–37	37–40
Serum concentrations						
Total cholesterol mmol/l	6 3±1 3 (46)	5 5*±1 5 (12)	5 1**±0 9 (14)	1 9±0 4 (51)	2 2+0 6 (10)	2 4*±0 8 (14)
Triglycerides mmol/l	1 90±0 70 (46)	1 95±0 60 (13)	1 38**±0 50 (12)	0 29±0 19 (46)	0 21±0 11 (10)	0 77±0 27 (14)

Using Student s *t* test mean significantly different from that in term group **p<0 01 *p<0 05

Pearse for their suggestions and criticism Dr R G Pearse also corrected the English text

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Submitted Aug 31 1979
Accepted May 6 1980

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than that of SFD infants ($p<0.05$) Individual values in cord sera of T C for the 3 different groups are plotted against gestational age in Fig 1 (lower part) In the AGA babies, the individual T C concentrations varied little from 34 to 41 weeks gestation, whereas the concentrations in the SFD infants were generally higher than in the AGA babies Among the three groups of infants mean TG concentration at birth was higher in the term than in the preterm AGA infants (0 29 and 0 21 mmol/l respectively), but was highest in the SFD babies (0 77 mmol/l)

DISCUSSION

Human pregnancy is accompanied by maternal hypercholesterolaemia (16, 22) Isotope studies suggest that the increased maternal steroid production during pregnancy is derived from increased circulating cholesterol (14) Although in underdeveloped countries lower cholesterol values have been found in non-pregnant women (14), in this study at the end of normal pregnancy T-C values (6 3±1 3 mmol/l) did not differ notably from the range of means reported in Western countries 5 66 to 6 48 mmol/l by Kleeberg & Polishuk (18) Spellacy et al (24) Kesteloot et al (17) There is some evidence (12 16) that the level of T C declines or at least stops rising during the last stage of pregnancy in Caucasian mothers whereas in this study it seems to rise up to term and remained nearly constant after term (Fig 1) It remains uncertain whether this relatively greater increase in Tanzanian women during the last stage of pregnancy could indicate a different pattern of steroid production

Mean T C levels in cord blood from term AGA infants in this study (1 9±0 4 mmol/l) were comparable to the range of means reported from Western countries 1 58 to 2 49 mmol/l (5, 6, 8, 10, 11, 13, 17, 18) No notable differences were observed between males and females (1 92 and 1 87 mmol/l respectively) Our observations show that in AGA babies delivered beyond 34 weeks gestation, serum T-C concentrations remained virtually constant, similar to findings in Great Britain (10) and the USA (12), but are different from the results obtained by Andersen & Friis Hansen (1) Our finding of elevated cord serum T C values in SFD infants when compared to AGA infants remains unexplained It is probably as suggested by Cress et al (5) that effects of fetal stress on sympathic nervous system activity could lead to an increased mobilisation of adipose tissue

Maternal TG concentration remained virtually constant during the last stage of pregnancy in mothers delivering an AGA infant This is in agreement with observations in the USA by Friedman et al (12) The lower TG values in mothers giving birth to a SFD infant when compared to those who delivered an AGA infant remain unexplained

In agreement with the results of Fosbrooke & Wharton (10) and Andersen & Friis Hansen (1) cord blood TG values increased with gestation However highest values were found in SFD infants which could also be explained by an increased mobilisation of the fetal adipose tissue stores in response to intrauterine stress (5)

The overall significance of our observations in relation to maternal/cord blood lipids at delivery remains uncertain It seems that the concentration of these lipids were in general similar in this study and in Western countries during late pregnancy in the mother and her offspring However different levels were observed beyond this stage A similar tendency was observed between Tanzanian and West-European infants as regards the fatty acid composition of their body fat before and after birth (2)

ACKNOWLEDGEMENTS

This study was supported by a grant from the foundation De Drie Lichten I am grateful to the following for their great help and cooperation Mr H Visser Mrs P Visser Mr L Baars Dr E BenGershon and his staff Mrs M Boersma and Mrs T vd Laar I owe a great debt to Prof J H P Jonxis Prof H K A Visser and Dr R G

Acta Pædiatr Scand 69 753–760, 1980

PEPTIC ULCER DISEASE IN EARLY INFANCY CLINICAL PRESENTATION AND ROENTGENOGRAPHIC FEATURES

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ABSTRACT Johnson D, L'Heureux, P and Thompson, T. (Departments of Pediatrics and [illegible] of Minnesota School of Medicine, Minneapolis, Minnesota) Peptic [illegible] year period [illegible] bleeding and/or perforation commonly associated with ulcer disease in early infancy was present in only seven (44%) of the 16 patients The remaining nine infants (56%) presented with recurrent emesis, a presentation of peptic ulcer disease rarely described in the first weeks of life Contrast radiography was used to demonstrate a definite ulcer crate in 13 of these patients Two had their ulcers diagnosed at surgery and one by endoscopy. Radiographic demonstration of pylorospasm and/or gastric retention was often associated with ulcer craters located in the pyloric channel or gastric antrum Only two patients required surgery for massive bleeding and perforation The remainder were successfully managed medically using orogastric suction, antacids and gradual resumption of milk feedings There were no deaths within our population attributable to peptic ulcer disease.

KEY WORDS Peptic ulcer disease, infants, vomiting, gastric outlet obstruction, gastrointestinal hemorrhage

The existence of peptic ulceration in the newborn period and during early infancy has been recognized since the early 19th century (24) In contrast to the generally chronic course of the disease in older children and adults, ulceration in early infancy has been characterized by the precipitous onset of gastrointestinal bleeding, often with perforation Our data suggests that peptic ulcer disease in the first weeks of life may not only present acutely, but may more often present with recurrent vomiting This latter presentation has rarely been appreciated in the first weeks of life suggesting that peptic ulcer disease is a more common cause than previously recognized for recurrent vomiting in the neonatal period

SUBJECTS AND METHODS

During the seven-year period from February 1, 1971, to December 31 1978 16 infants (0 54% of admissions) hospitalized on the Newborn Intensive Care Unit at the University of Minnesota Hospitals had an ulcer crater unequivocally defined using contrast radiography and fluoroscopy (13 patients), endoscopy (1 patient) or surgery (2 patients) All infants with the exception of the two who showed signs of perforation, were treated medically Medical treatment consisted of discontinuation of feedings and initiation of intermittent orogastric suction Ongoing blood losses were replaced when patients with bleeding ulcers were deficit 10% of their total blood volume or exhibited hypotension Every 2 hours, 3–5 ml of magnesium–aluminium hydroxide antacid was placed in the stomach and the orogastric tube was clamped for 30 min After 48 hours, feedings were initiated using 10 ml of 17-calorie per dl formula or breast milk fed by gavage every four hours Feedings were then gradually increased to volumes and caloric strengths previously tolerated by the infants Following initiation of feedings the same dose of antacid was gavaged between feedings This treatment was continued for four weeks

RESULTS

Clinical presentation

The clinical data (Table 1) showed an equal number of male and female, and of full term

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PEPTIC ULCER DISEASE IN EARLY INFANCY: CLINICAL PRESENTATION AND ROENTGENOGRAPHIC FEATURES

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Table 1 *Clinical data and hospital course of 16 infants with peptic ulcer disease*

AGA = appropriate for gestational age SGA = small for gestational age HMD = hyaline membrane disease BPD = bronchopulmonary dysplasia DIC = disseminated intravascular coagulation PDA = patent ductus arteriosus NEC = necrotizing enterocolitis H = hematemasis P = perforation CR = chronic regurgitation UGI = upper gastrointestinal contrast radiography END = endoscopy LC = lesser curve of the stomach D = duodenum PC = pyloric channel M = medical SC = surgical closure

Patient	Sex	Birth weight (g)	Gestational age: Weeks	Gestational age: Growth	Apgars: 1	Apgars: 5	Perinatal complications	Onset of symptoms (day of age)	Initial symptoms	Day of diagnosis
1	F	3 420	37	AGA	6	8	*None*	2	H	3
2	M	2 450	34	AGA	7	7	2nd of twins HMD	4	H	4
3	M	1 645	35	SGA	8	8	Hypoglycemia	3	H	3
4	F	2 210	40	SGA	9	9	None	1	H	[illegible]
5	M	3 240	40	AGA	–	–	None	3	H	4
6	F	1 956	32	AGA	9	10	1st of twins HMD BPD cor pulmonalae subglottic stenosis	66	H+P	66
7	M	4 090	43	AGA	8	8	Fetal distress meconium aspiration syndrome	6	H+P	6
8	M	3 700	40	AGA	7	9	Pneumonitis hyperbilirubinemia	7	CR	10
9	M	3 600	40	AGA	6	8	Aortic stenosis	14	CR	17
10	F	2 190	35	AGA	4	6	HMD	24	CR	43
11	M	1 560	32	AGA	1	5	HMD PDA DIC BPD perinatal asphyxia NEC seizures Staph sepsis	33	CR	0
12	F	1 990	35	AGA	1	4	Perinatal asphyxia HMD DIC hyperbilirubinemia	16	CR	35
13	M	3 300	40	AGA	9	10	*E coli* sepsis	12	CR	19
14	F	3 100	40	AGA	5	7	Right hydronephrosis left pelvic kidney seizures and hypotonia bilateral hip dislocation	10	CR	14
15	F	2 675	38	AGA		–	Pneumonitis NEC with perforation Candida skin abscess enterococcal sepsis	58	CR	8
16	F	1 658	34	AGA	9	9	Onset of vomiting with the first *feeding*	1	CR	12

[a] Normal gastrin levels
1 day of age (6) *100±13 pg/ml*
4 days of age (13) 151±15 8 pg/ml
1 1? days of age (17) 130 4+11 4 pg/ml
1 5–?? months of age (17) 101 4+6 6 pg/ml
3 wks 16 wks of age (14) 193+28 pg/ml

and premature infants Comparison of the full term and premature infants revealed no differences in ulcer locations or presenting symptoms No consistent association was noted between the mode of feeding or the use of intravenous hyperalimentation in the development of ulcers in our patients

Seven infants (44%), patients one through seven, presented acutely with symptoms of bleeding Nine infants (56%), patients eight through sixteen, presented with chronic recurrent vomiting The initial onset of symptoms in infants presenting with emesis occurred in a relatively even distribution during the first ten weeks of life (Fig 1*a*) With the exception of patient 16 symptoms in this group of infants began in the convalescent period some time after their perinatal complications Conversely the majority of infants (85%) who had gastrointestinal bleeding from ulcer disease presented during the first week of life (Fig 1*b*)

Diagnosis

Infants who presented with bleeding or bleeding with perforation had a definite diagnosis within 24 hours from the onset of symptoms

...de of ...gnosis	Location of ulcer	Rx	Comment
...D	LC+D	M	
...GI	LC	M	
...GI	LC	M	4 hour fasting gastrin = 126 pg/ml day 8ª
GI	LC	M	4 hour fasting gastrin 96 pg/ml day 3ª
GI	LC	M	
urg	D	SC	High dose steroids for BPD
...rg	D	SC	5 days of IV tolazoline
GI	LC	M	
GI	LC	M	Problem noted postvalvotomy
GI	LC	M	
LGI	Post wall antrum	M	
LGI	PC	M	4 hour fasting gastrin =481 pg/ml day 36ª
LGI	PC	M	
LGI	PC	M	
LGI	PC	M	Atropine 0 02 mg/kg given q i d for persistent pylorospasm
LGI	PC	M	A lactobezoar was also documented on LGI on day 12

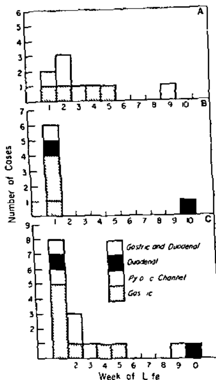


Fig 1 Time of presentation location of ulcer and symptomatology in the 16 infants with peptic ulcer disease (*a*) Time of presentation and ulcer location in infants presenting with vomiting (*b*) Time of presentation and ulcer location in infants presenting with gastrointestinal bleeding (*c*) Time of presentation and ulcer location in all 16 infants

Among our patients with recurrent vomiting, the diagnosis of peptic ulcer disease was often delayed (mean 11 4 days range 3–20)

Most patients (81 %) in our series had their ulcer craters defined using barium contrast radiography with fluoroscopy Table 2 details the roentgenographic findings in these 13 patients Figs 2 3 and 4 illustrate the typical roentgenographic appearance of ulcer craters of the pyloric channel, of the lesser curvature, and of the posterior antral wall, respectively Seventy eight per cent of the infants who presented with recurrent vomiting demonstrated pylorospasm This observation was independent of either a gastric or pyloric ulcer location Gastric outlet obstruction, documented radiographically by a markedly dilated stomach containing large amounts of residual food and secretions and pylorospasm, occurred together in 80% of the infants with pyloric channel ulcers Only one of the four infants who presented with bleeding had similar roentgenographic findings

Periodic sampling of stool and gastric aspirates specimens for guaiac positivity was conducted on all infants Only three of the nine infants (patients 8, 13 and 15) who presented with recurrent emesis had guaiac positive stools, emesis, or gastric aspirates In all three infants, the positivity appeared long after the onset of symptoms

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AGA = appropriate for gestational age SGA = small for gestational age HMD = hyaline membrane disease BPD = bronchopulmonary dysplasia DIC = disseminated intravascular coagulation PDA = patent ductus arteriosus NEC = necrotizing enterocolitis H = hematemasis P = *perforation* CR = chronic regurgitation UGI = upper gastrointestinal contrast radiography END = endoscopy LC = *lesser curve of the stomach* D = duodenum PC = pyloric channel medical SC = surgical closure

Patient	Sex	Birth weight (g)	Gestational age		Apgars		Perinatal complications	Onset of symptoms (day of age)	Initial symptoms	Day diag nosi
			Weeks	*Growth*	*1*	*5*				
1	F	3 420	37	AGA	6	8	None	2	H	3
2	M	2 450	34	AGA	7	7	2nd of twins HMD	4	H	4
3	M	1 645	35	*SGA*	*8*	*8*	*Hypoglycemia*	3	H	3
4	F	2 210	40	SGA	9	9	None	1	*H*	2
5	M	3 240	40	AGA	–	–	None	3	H	4
6	F	1 956	32	AGA	9	*10*	1st of twins HMD BPD cor pulmonalae subglottic stenosis	66	H+P	66
7	M	4 090	43	AGA	*8*	*8*	Fetal distress meconium aspiration syndrome	6	H+P	6
8	M	3 700	40	AGA	7	9	Pneumonitis hyperbilirubinemia	7	CR	10
9	M	3 600	40	AGA	6	*8*	Aortic stenosis	14	CR	17
10	F	2 190	35	AGA	4	6	HMD	24	CR	43
11	M	1 560	32	AGA	*1*	5	HMD PDA DIC BPD perinatal asphyxia NEC seizures Staph sepsis	33	CR	50
12	F	1 990	35	AGA	*1*	4	Perinatal asphyxia HMD DIC hyperbilirubinemia	16	CR	35
13	M	3 300	40	AGA	9	*10*	*E coli* sepsis	12	CR	19
14	F	3 100	40	AGA	5	7	Right hydronephrosis left pelvic kidney seizures and hypotonia bilateral hip dislocation	10	CR	14
15	F	2 675	38	AGA	–	–	Pneumonitis NEC with perforation Candida skin abscess enterococcal sepsis	58	CR	78
16	F	1 658	34	AGA	9	9	Onset of vomiting with the first feeding	1	CR	12

* Normal gastrin levels
1 day of age (6) 100±13 pg/ml
4 days of age (13) 151±15 8 pg/ml
1 12 days of age (17) 130 4±11 4 pg/ml
1 5–22 months of age (17) 101 4+6 6 pg/ml
3 wks 16 wks of age (14) 193±28 pg/ml

and premature infants Comparison of the full term and premature infants revealed no differences in ulcer locations or presenting symptoms No consistent association was noted between the mode of feeding or the use of intravenous hyperalimentation in the development of ulcers in our patients

Seven infants (44%), patients one through seven, presented acutely with symptoms of bleeding Nine infants (56%), patients eight through sixteen, presented with chronic recurrent vomiting The initial onset of symptoms in infants presenting with emesis occurred in a relatively even distribution during the first ten weeks of life (Fig 1*a*) With the exception of patient 16 symptoms in this group of infants began in the convalescent period some time after their perinatal complications Conversely the majority of infants (85%) who had gastrointestinal bleeding from ulcer disease presented during the first week of life (Fig 1*b*)

Diagnosis

Infants who presented with bleeding or bleeding with perforation had a definite diagnosis within 24 hours from the onset of symptoms

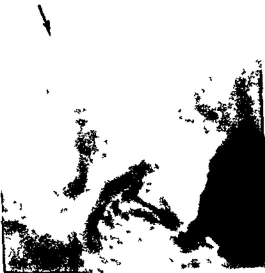


Fig 3 A characteristic lesser curvature ulcer of a gastric antrum is identified The stomach is not dilated and there is immediate exit of barium into the duodenum



Fig 4 A large triangular shaped ulcer crater is identified on the posterior wall of the gastric antrum Note the surrounding halo of mucosal edema

ally with symptoms of obstruction rather than bleeding or perforation and five of these nine patients presented with vomiting within the first two weeks of life This is a much higher percentage of patients with symptoms of ob struction presenting early in the newborn period than has previously been reported (Table 3) The delay in diagnosis noted among the infants presenting with recurrent vomiting probably resulted from failing to recognize this *symptom as an initial presentation of ulcer* disease in the first weeks of life

One interesting aspect of our series is the high incidence of ulcers located in the pyloric channel Ulcers in this location have rarely been mentioned as occurring in infancy Bird et al (1) and Thompson & Jewett (22) included them in their duodenal ulcer group, but did not comment upon them further (Table 2) Grossfeldt et al (7) reported a five-week-old female who had vomiting since birth and was found to have a pyloric channel ulcer by endoscopy Pyloric channel ulcers in adults are not rare (16) Affected adults often present with a specific clinically recognizable "pyloric syndrome" consisting of nausea and vomiting, pain soon after meals, and weight loss Our patients with pyloric channel ulvers presented with a very similar "pyloric syndrome" charakterized by recurrent vomiting, feeding intolerance and/or poor weight gain Radiographically our infants demonstrated the same findings as reported in adults a pyloric ulcer crater and evidence of gastric outlet obstruction (11)

Diagnostic evaluation involving more than simple screening for occult blood should be considered in all infants who present with major gastrointestinal bleeding or with chronic symptoms of gastric outlet obstruction Contrast radiography appears to be a useful and well tolerated procedure for diagnosing ulcer disease in infants This technique can be made even more effective by recognizing the frequent association of ulcer disease with pylorospasm and gastric outlet obstruction This association, *first discussed by Holt* (9) was present in a high proportion of our infants,

Table 2 *Roentgenographic findings in the 13 patients diagnosed as having an ulcer crater using barium contrast radiography*

Location	Patient number	Pyloro spasm	Outlet obstruction	Gastro-esophageal reflux	Presentation Vomiting	Presentation Bleeding
Pylorus	13	+	+		+	
	15	+	+		+	
	16	+	+		+	
	14	+	+	+	+	
	12			+	+	
Lesser curve	8	+			+	
	9	+			+	
	10	+			+	
	2	+	+	+		+
	3					+
	4					+
	5					+
Post wall antrum	11				+	

+ = present

Outcome

Patients six and seven were surgically explored because of intractable gastrointestinal bleeding and radiographic evidence of perforation The remainder were successfully managed medically Hemorrhage ceased within 24 hours in all medically treated infants with bleeding ulcers and only a minority of patients required urgent volume expansion and blood replacement Feedings instituted following the short period of gastric drainage and antacid administration were well tolerated in all infants except for patient 15 No alkalosis secondary to the antacids was observed No patients experienced recurrence of symptoms

felt to be necessary There were no deaths in our series attributable to peptic ulcer disease



Fig 2 The stomach is markedly dilated with marked delay in gastric emptying The pylorus is spastic and a persistent small ulcer niche can be identified (arrow)

DISCUSSION

Peptic ulcer disease in early infancy was recognized by von Siebold (24) who reported a perforated gastric ulcer at autopsy in a two day old infant Bird et al (1) in 1943 reviewed 243 cases of peptic ulcer disease in children and noted that ulcers in the newborn and early infancy bled and/or perforated Several authors have recognized recurrent vomiting as a frequent presenting symptom of peptic ulcers in older children (2 5) but only two infants less than two weeks of age have been reported with this presentation (1,10) (Table 3) In contrast, nine of our 16 patients (56%) presented initi

ic ulcer location

tric	Duodenal	Duodenal and pyloric	Pyloric channel	Gastric and duodenal
		26		
		43		
				1
	7			
	2			
	2			
	4			
	6			
		3		
		3		
	2			
	11			
1	8			
	34			
3	5			
5	27			
	5			
5	1		3	1
2	1		2	

ment with gastric drainage, antacids, and slow resumption of milk feedings was successful in managing 13 of our 14 medically treated patients One infant, patient 15, required the addition of atropine for persistent pylorospasm

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1 Bird D E., Limper, M A & Mayer, J M Surgery in peptic ulceration of stomach and duodenum in infants and children *Ann Surg, 114* 426 1941.

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21 Stanley Brown, E G & Stevenson S S Massive gastrointestinal hemorrhage in the newborn infant *Pediatrics, 35* 482, 1965

22 Thomson, N B & Jewett, T C Peptic ulcers in infancy and childhood *JAMA 189* 539, 1964

Table 3 *A composite review of literature including the present series detailing clinical presentation sex and lesion location in infants with peptic ulcer disease presenting at 0–15 days a[nd] 15 days to one year of life*

Author and year	No of cases	Presentation: Vomiting	Perforation	Bleeding	Perforation and bleeding	Other	Sex: Male	Female	Unknown
1 Bird et al (1941)	243								
0–14 days	38	1	18	22		1	15	11	12
15 days–1 year	51	8	15	25		6	22	15	14
8 Guthrie (1942)	9								
0–14 days	1			1				1	
15 days–1 year	7	5		2			4	3	
18 Schuster & Gross (1963)	28								
0–14 days	2			1	1		2		
15 days–1 year	3	2		1			2	1	
10 Leix & Greaney (1963)	68								
0–14 days	10	1	2	5	1	1	5	5	
15 days–1 year	11			6	3	2	8	3	
22 Thomson & Jewett (1964)	50								
0–14 days	6		4		2		3	3	
15 days 1 year	4		3	1			2	2	
20 Spencer (1964)	54								
0–14 days	11		5	2	2	2			11
15 days–1 year	15			8	7				15
12 Raffensperger et al (1966)	60								
0–14 days	21		17	4					21
15 days–1 year	34		18	16					34
23 Tudor (1967)	409								
0–14 days	8						5	3	
15 days–2 years	33						21	12	
15 Rosenlund & Koop (1970)	27								
0–14 days									
15 days–1 year	5	1		1	3		4	1	
Present series (1979)	16								
0–14 days	11	5		5	1		7	4	
15 days–3 months	5	4			1		1	4	

especially those with symptoms of recurrent vomiting When this was noted special care was taken to completely visualize the pyloric channel and distal gastric antrum in order to detect all ulcer craters

Endoscopy, though not as readily available as contrast radiography is an excellent technique which can be used in many infants Endoscopy may be especially helpful in infants who present with bleeding since contrast radiography has been reported to detect a bleeding source in only one third to one half of the patients (19) whereas endoscopy is 84% effective (3)

Early reports of peptic ulcer disease in infancy emphasized the high mortality rate among the affected individuals and stated that surgery offered the best hope of survival (1 8) With improved techniwue of infant support conservative medical management with close attention to adequate blood replacement can be successfully employed in almost all infants with the exception of those who show evidence of perforation (4 7 21) Medical treat

eptic ulcer location				
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12		26		
8		43		
				1
	7			
	2			
1	2			
6	4			
5	6			
		3		
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	11			
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	5			
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…tric	Duodenal	Duodenal and pyloric	Pyloric channel	Gastric and duodenal
		26		
		43		
				1
	7			
	2			
	2			
	4			
	6			
		3		
		3		
[illegible]	2			
[illegible]	11			
[illegible]	8			
	34			
	5			
	27			
	5			
	1		3	1
	1		2	

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Submitted Jan 24, 1980
Accepted May 20, 1980

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Acta Pædiatr Scand 69 761–770 1980

PULMONARY MECHANICS, CHEST X RAY AND LUNG DISEASE AFTER MECHANICAL VENTILATION IN LOW BIRTH WEIGHT INFANTS

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ABSTRACT Lindroth, M , Jonson, B , Svenningsen, N W and Mortensson, W (Neonatal Unit, Department of Paediatrics, University Hospital, Lund, Sweden) Pulmonary mechanics, chest X ray and lung disease after mechanical ventilation in low birth weight infants Acta Paediatr Scand, 69 761, 1980 —Pulmonary mechanics, chest X ray and the incidence of [illegible] 41 low birth weight infants treated with intermittent [illegible]

[illegible] life but subsided later on Development of BPD or later respiratory [illegible] related to treatment with high inspired oxygen concentrations but commonest in patients with hyaline membrane disease The combined findings of pulmonary mechanics and chest X ray shortly after IPPV were correlated to later clinical lung disease

KEY WORDS Newborn infants, IPPV, follow up study, pulmonary mechanics, bronchopulmonary dysplasia, chest X ray

Intermittent positive pressure ventilation (IPPV) is increasingly used in neonatal intensive care Recurrent bronchitis and pneumonia are common during the first years of life after IPPV (8 15 16 20) as are abnormal chest X rays (8 10, 13 24, 31) and lung function tests (2 8 20 34) The aim of the present study was to study chest X ray and pulmonary mechanics in IPPV survivors, and to relate these findings to clinical lung disease during the first years of life

METHODS

Lung mechanics was studied as previously described (1 21) The flow and pressure signals from a body plethysmograph and an oesophageal catheter were transmitted to a computer that calculated breathing frequency f, tidal volume V_T minute ventilation $\dot{V}_E$ dynamic compliance C_{dyn} and functional pulmonary resistance R_F The computer also drew pressure flow loops for visual interpretation and for calculation of resistance at zero flow at the end of expiration R_{ee} (1)

The *chest radiographs* at the times for the pulmonary mechanics examinations were reviewed by W M without knowledge of original diagnosis or duration of IPPV The radiographs were classified according to the predominant finding in one of 5 groups 1) "Normal , 2) BPD = small rounded areas of radiolucency alternating with thin strands of radiodensity i e BPD stage IV according to Northway et al (24) 3) Hazy = indistinct infiltration around vessels and bronchi close to and in the pulmonary hili 4) Overdistention = overdistended lungs with increased radiolucency 5) Others including local lung overdistention and atelectasis (Fig 1)

Patients

From January 1976 to June 1978 48 low birth weight (LBW) infants (<2 5 kg) survived after IPPV at our Neonatal Intensive Care Unit (NICU) Seven infants IPPV treated 1 to 7 days were excluded from the study because early examination with pulmonary mechanics was not performed

Hyaline membrane disease (HMD) was diagnosed in patients 1–28 on diagnostic criteria described elsewhere (19) In 10 patients (Nos 29–38) IPPV was started because of *apnoea repetens (AR)* diagnosed on the basis of recur

Supported by grants from the Swedish Medical Research Council (No 29X-4732 and 14X-02872) and the Swedish National Association against Chest and Heart Diseases



Fig 1 (a) BPD slight overdiste tion and general interstitial pare chymal changes (b) Hazy sl gh indistinct interstitial changes clo to the hili In addition a pneumc process in the middle lobe (c) O distention at 11 months of age

rent apnoeas with bradycardia without signs of infection in infants usually with a gestational age of less than 32 weeks and a normal chest X ray Another 3 patients (Nos 39–41) were IPPV treated because of septicemia chylo thorax and pneumonia Clinical data for the different di agnostic groups are given in Table 1

During the study period 31 LBW infants died all of which were IPPV treated Eight of them were ventilated for more than 7 days and 4 developed BPD which was verified at autopsy In one case BPD was the probable cause of death

IPPV Indications for IPPV were recurrent apnoeas with bradycardia persistent severe hypercarbia and/or persistent severe hypoxia in spite of high inspired oxygen c

a cu

2 5–3 0) No infant was tracheotomized . . used were Servo Ventilator 900 or 900 B (Siemens Elema AB Solna Sweden) or Loosco Amsterdam Ventilator Mk 1 and Mk 2 (Loos & Co Amsterdam The Netherlands) The usual initial settings for HMD were peak pressure 20–24 cm H_2O positive end expiratory pressure (PEI 2–4 cm H_2O inspiratory expiratory ratio 1 1 frequei 40 per min and inspired oxygen concentration 40% I AR and septicemia the settings were peak pressure 15 H_2O no PEEP inspiration expiration 1 1 or less 1 quency 40 and oxygen concentration 30% or less Blc gas controls and other routines during the intensive ca period have been described in detail elsewhere (19)

The first combined exam nation with pulmonary n chanics and chest X ray was performed when the patiei were still at the NICU The median time interval betwe end of assisted ventilation and examination was 11 da with a range of 2–77 days (Fig 2) Twenty n ne patiei were reinvest gated during the first ? years because abnormal previous findings or very long IPPV treatmei Individual values were regarded as abnormal if they fi outside the 95% confidence limits for predicted value For group comparisons the observed individual valu were compared to the one expected from body length (21

To evaluate the prognostic value of chest X ray and pu monary mechanics shortly after IPPV a predictive scoi was calculated BPD changes on chest X ray were give

Table 1 *Clinical data of 41 patients IPPV treated for hyaline membrane disease (HMD), apnoea repetens of immaturity (AR) or other diseases (median and range)*

	N	Birth weight (kg)	Gest age (weeks)	IPPV (days)
HMD	28	1 2 (0 8–2 4)	29 (27–36)	24 5 (5–72)
AR	10	1 0 (0 8–1 6)	29 (26–33)	5 5 (2–58)
Others	3	1 4 (0 9–1 5)	28 (28–34)	6 (6–8)
All	41	1 2 (0 8–2 4)	29 (26–36)	15 (2–72)

2 points other abnormalities 1 and normal radiographs zero points For pulmonary mechanics the C_{dyn} and R_{eE} values were found most suitable C_{dyn} values higher and R_{eE} values lower than expected were counted as zero Deviations in the opposite directions were expressed in standard deviations and given corresponding points signs ignored to the score Thus a R_{eE} value 2 5 S D higher or C_{dyn} 2 5 S D lower than expected gave 2 5 points The predictive score for each patient was the sum of the radiographic and lung function points

The number of pneumonias verified radiographically and bronchitis with wheezing and rales were registered for all infants In order to quantify clinical lung morbidity the patients received a respiratory disease score for the first 2 years Each pneumonia or bronchitis requiring hospitalization gave 3 points and each bronchitis treated at home 1 point Patients with 3 or more points were arbitrarily classified as diseased the others as healthy

Statistics

Student s two-tailed *t* test and Mann Whitney s rank sum test were used to examine differences between groups Probability values of $p<0.05$ $p<0.01$ and $p<0.001$ are indicated as * ** and ***, respectively

RESULTS

Clinical data, chest X ray, pulmonary mechanics findings and ages at the first and latest examination for each individual are presented elsewhere (22)

Roentgenographic examination of the lungs

The time interval between the end of assisted ventilation and the first examination of lung function and chest X ray varied widely (Fig 2) The median time interval in the different radiographic groups, however, did not differ significantly At the first examination 19 infants had normal and 22 abnormal chest X-ray (Fig 2) From Table 2 it appears that patients with normal radiographs had higher birth weight and shorter duration of IPPV than BPD patients Patients with normal X-ray had significantly lower compliance and higher resistance than expected but the BPD patients had still more abnormal lung mechanics data Eleven out of 19 infants with normal radiographs had had HMD compared to 8 out of 10 BPD patients Thus it appears that the typical BPD patient was of very low birth weight, had suffered from HMD and been treated for a long time with IPPV In Table 2 values for infants with perihilar haziness lay between those with normal X ray and those with BPD in all respects

Follow-up examination with late X-ray at

Table 2 *Clinical data and deviation from expected values on pulmonary mechanics in 36 patients with normal radiographs BPD or perihilar haziness on chest X-ray shortly after IPPV (median and range)*

Chest X ray	N	Birth weight (kg)	Duration of IPPV (days)	Disease score	Deviation from expected (in standard deviations) C_{dyn}	R_t	R_{eE}
Normal	19	1 4 (0 8–2 4)	8 (2–66)	1 4 (0–12)	−1 7 (0 5–−3 3)	1 8 (−2 3–5 5)	1 3 (−3 0–3 3)
BPD	10	1 0 (0 8–1 5)**	32 (13–59)**	7 5 (0–22)*	−2 6 (−0 2–−3 5)	3 5 (−1 6–6 2)	2 9 (0–5 5)
Hazy	7	1 3 (0 9–2 4)	9 (5–72)	3 (0–6)	−2 3 (0 7–−3 1)	1 8 (−1 4–2 6)	2 0 (−0 7–3 0)

* Significantly different from patients with normal chest X ray

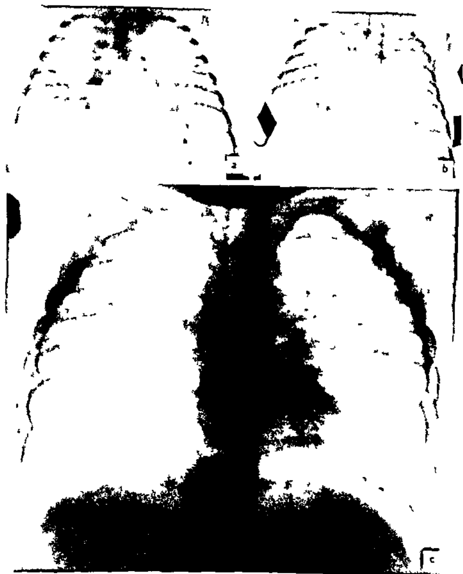


Fig 1 (a) BPD slight overdistention and general interstitial parenchymal changes (b) Hazy slight indistinct interstitial changes close to the hili In addition a pneumonic process in the middle lobe (c) Over distention at 11 months of age

rent apnoeas with bradycardia without signs of infection in infants usually with a gestational age of less than 32 weeks and a normal chest X ray Another 3 patients (Nos 39–41) were IPPV treated because of septicemia chylothorax and pneumonia Clinical data for the different diagnostic groups are given in Table 1

During the study period 31 LBW infants died all of which were IPPV treated Eight of them were ventilated for more than 7 days and 4 developed BPD which was verified at autopsy In one case BPD was the probable c... of death

... com...

T... ated ... 2 5–3 0) No infant was tracheotomized ... used were Servo Ventilator 900 or 900 B (Siemens Elema AB Solna Sweden) or Loosco Amsterdam Ventilator Mk 1 and Mk 2 (Loos & Co , Amsterdam The Netherlands) The usual initial settings for HMD were peak pressure 20–24 cm H_2O positive end expiratory pressure (PEEP) 2–4 cm H_2O inspiratory expiratory ratio 1 1 frequency 40 per min and inspired oxygen concentration 40% For AR and septicemia the settings were peak pressure 15 cm H_2O no PEEP inspiration expiration 1 1 or less frequency 40 and oxygen concentration 30% or less Blood gas controls and other routines during the intensive care period have been described in detail elsewhere (19)

The first combined examination with pulmonary mechanics and chest X ray was performed when the patients were still at the NICU The median time interval between end of assisted ventilation and examination was 11 days with a range of 2–77 days (Fig 2) Twenty nine patients were reinvestigated during the first 2 years because of abnormal previous findings or very long IPPV treatment

To evaluate the prognostic value of chest X ray and pulmonary mechanics shortly after IPPV a predictive score was calculated BPD changes on chest X ray were given

Table 3 *Radiographic findings in 25 patients shortly after IPPV and at follow up at 1/2 to 2 years of age*

Late x ray	Initial X ray Normal	BPD	Hazy	Others	Total
Normal	2	1	1	1	5
BPD	0	3	0	0	3
Overdistention	5	5	3	3	16
Others	0	0	1	0	1
Total	7	9	5	4	25

Dynamic compliance C_{dyn}, was only 50% of expected normal values at the first examination (Table 4 and Fig 4) At follow up examination all values were within the normal range although the patients as a group still had lower than expected C_{dyn}

Pulmonary resistance R_t, was very much increased (Table 4) Most infants with abnormally high R_t at the first examination normalized but 7 HMD patients had persistingly abnormal R_t at the latest examination (Fig 5) All these patients had clinical lung disease

End expiratory resistance R_{eE}, was more increased than R_t both at the first and latest examinations (Table 4) Abnormally high R_{eE} was found in 20 patients at the first and 4 at the latest examination With one exception abnormally high R_{eE} coincided with abnormally high R_t

Signs of upper airways obstruction appearing on the pressure flow loops as hampered inspiratory flow were not present in any case at the first examination At reinvestigation this loop pattern was found in one patient who, however, had an acute rhinitis on this occasion

Clinical lung disease

Most patients had pneumonia or bronchitis during the first years of life (Fig 2) The HMD patients had a median disease score of 4 7 compared to zero in non HMD patients However, 7 HMD patients IPPV treated for more than 14 days had a disease score below 3 Six of these infants were born during the latter half of the study period

The capability of the first examination with pulmonary mechanics and chest X-ray to predict later lung morbidity was tested by comparing predictive scores to lung disease scores (Fig 6)

Only 2 patients with a predictive score below 4 had disease score ≥3 Both these patients (Nos 28 and 41) had parents and siblings with bronchial asthma and other allergic diseases

If we predict that subjects with a predictive score ≥4 will show up with clinical lung disease the sensitivity and specificity was 0 91 and 0 79, respectively, and the predictive value for positive and negative tests 0 83 and 0 88, respectively (23)

Oxygen concentrations and peak pressures

In HMD the BPD patients had a longer median duration of IPPV but shorter time in high inspired oxygen concentration (≥40%) than those with normal chest X ray (Table 5) These differences were statistically not significant Two BPD patients (Nos 12 and 32) never received high oxygen concentrations whereas

Table 4 *Percentage of expected values of some pulmonary functions in IPPV treated infants at the first and latest examination with pulmonary mechanics*

	f	V_{iE}	V_T	C_{dyn}	R_t	R_{eE}
1st examination % ($N = 41$)	108	99	90	50***	217***	273***
Re-examination % ($N = 29$)	110	116*	105	89**	145***	167**

Pat No	Diagnosis	Birth weight kg	IPPV days	Duration of follow-up (years) 1 2	Deviation from expected (SD) C_{dyn} R_f R_{eE}	Initial X ray
1	HMD	8	32		2 8 3 6 6	Normal (16)
2	HMD	8	66		1 4 2 1 1	Normal (50)
3	HMD	9	35		1 7 3 8 3 0	B D (7)
4	HMD	9	19		2 1 6 8	BPD (7)
5	HMD	9	72		9 6 2 3	Hazy (4)
6	HMD	9	9		1 6 1 1 5	B D (15)
7	HMD	9	29		3 5 4 3 0	B D (9)
8	HMD	1 0	6		1 0 3 8	thers (77)
9	HMD	1 0	36		2 6 4 4 2 7	BPD (4)
10	HMD	1 1	8		0 3 4 3 3	Normal (4)
11	HMD	1 1	7		1 4 5 3 3 0	Normal (18)
12	HMD	1 1	13		3 5 6 2 5 3	BPD (16)
13	HMD	1 1	4		2 2 6 2 5 5	BPD (17)
14	HMD	1 2	33		2 8 2 1 2 1	Others (23)
15	HMD	1 2	15		2 0 3 9 4 8	Others (15)
16	HMD	1 2	22		2 3 1 4 7	Hazy (2)
17	HMD	1 3	5	†	1 9 3 3 3 1	No mal (22)
18	HMD	1 3	48		3 3 1 2 3 1	Normal (18)
19	HMD	1 3	15		3 1 2 3 3 0	Hazy (6)
20	HMD	1 4	5		2 9 2 1 8	Normal (5)
21	HMD	1 4	36		3 7 4 6 4 9	Others (10)
22	HMD	1 5	35		2 5 1 9	B D (11)
23	HMD	1 6	15		2 3 4 2 9	Normal (5)
24	HMD	1 6	14		1 3 8 1 1	Normal (6)
25	HMD	1 9	4		2 1 1 4 2 0	Hazy (31)
26	HMD	2 3	7		2 8 1 5 3 0	Normal (4)
27	HMD	2 4	6		2 4 1 6 1 4	Hazy (7)
28	HMD	2 4	9		1 7 2 0 1 5	Normal (9)
29	AR	8	59		3 0 2 8 3 9	BPD (24
30	AR	1 0	9		2 1 2 6 2 6	Hazy (3
31	AR	1 0	3		3 1 3 1 6	Othe s (2
32	AR	1 0	4		2 8 3 1 2 6	BPD (6
33	AR	1 1	5		7 1 6 3	Hazy (14
34	AR	1 1	19		2 5 3 0	Normal
35	AR	1 3	5		1 5 1 6	ormal 14
36	AR	1 4	6		2 3 2 1 2	Normal 36
37	AR	1 5	3		5 2 7	ormal 9
38	AR	1 6	2		1 2 1 1	orma 14
39	Sept em a	1 4	9		4 8	Norma 3
40	Chyloth	9	6		5	Norma 6
41	Pne m n a	1 5	6		1	No a

Fig 2 Individual clinical course and findings for pulmonary mechanics and chest X ray at the first combined examination after IPPV Dynamic compliance (C_{dyn}) pulmonary resistance (R_f) and end expiratory resistance (R_{eE}) are expressed in standard deviations (S D) from expected value according to regression equation (21) Lung disease treated in hospital (▮) and at home (▏) Days between ended assisted ventilation and examination within parentheses

ages of 6 months or more was performed in 25 patients The radiographic findings shortly after IPPV and at follow-up in these cases are compared in Table 3 One BPD patient (No 4) already had normal X ray and pulmonary mechanics 2 weeks after the first examination Five patients with normal radiographs on the first occasion showed overdistention at ages of 8 months or more Patients with overdistention did not differ from those with normal late radiographs as regards duration of IPPV pulmonary mechanics or disease score

At the time for the first pulmonary mechanics examination 18 out of 41 patients were treated with 25–35% oxygen At follow-up examination no patient was oxygen dependent

Pulmonary mechanics

The findings of some pulmonary parameters expressed as percentages of expected values are given in Table 4

Breathing frequency minute ventilation and tidal volume The values of f V_T and V_{IE} at rest did not differ from expected normal values (Table 4) and all but few individual values were within the normal range (Fig 3) At follow up examination V_{IE} was higher than expected (Table 4) This increase was not correlated to overdistention on chest X ray

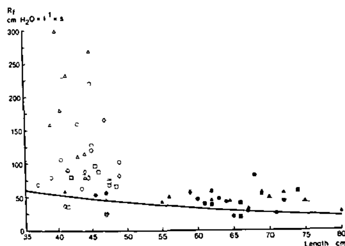


Fig 5 Pulmonary resistance (R_f) in relation to length in 41 IPPV treated patients Symbols see Fig 3

normal maturation of gas exchanging air spaces which takes place in the last trimester (29, 35)

Most studies of pulmonary sequelae after IPPV in preterm infants have dealt with the incidence and etiology of BPD (8 10, 13, 20, 24 25) In the initial study on BPD Northway et al correlated X ray stages of BPD to morphologic lung changes (24)

In the present study dynamic compliance was lower and pulmonary resistance higher than expected in nearly all infants (Fig 2) The deviation from expected values was largest in BPD patients but also present at other X ray changes or normal radiographs Obviously most of our IPPV patients acquired some lung damage in connection with the original disease or its treatment Probably the high resistance reflects morphological changes of small airways and the low compliance changes in other structures, e g in saccules If so, a majority of our patients, irrespective of X-ray findings, had bronchopulmonary dysplasia, the term used in a linguistic proper sense and the various X-ray changes would then only represent differences in degree

In the present material chest X-ray and pulmonary mechanics findings combined in a predictive score was better correlated to later

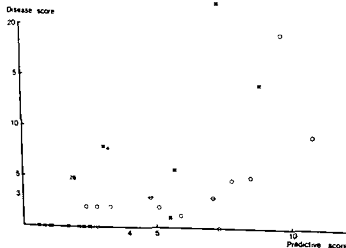


Fig 6 Clinical lung diseases during the first 2 years (disease score) in relation to predictive score based on the first combined examination with pulmonary mechanics and chest X ray The dashed lines delimit diseased infants (disease score ≥3) from healthy and those predictive to be diseased (predictive score ≥4) from those predictive to be healthy Two patients with strong heredity for allergic diseases are indicated with numbers

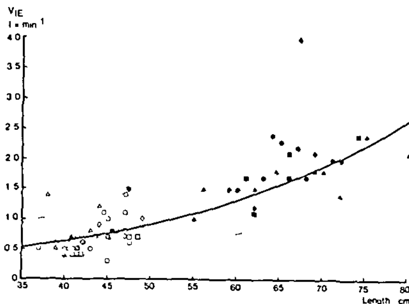


Fig 3 Minute ventilation (V_{IE}) at the first and latest examination in relation to length in 41 IPPV treated patients with various chest X ray findings on the first occasion Solid line indicates the expected value and the dashed lines the 95% confidence limits of individual observations for normal infants (21) Open symbols represent initial study, closed symbols represent latest examination O, Normal chest X ray Δ BPD □ perihilar haziness □ Other X ray changes

patients 14 and 18 did so for more than 3 weeks during IPPV without developing BPD

In HMD patients disease score, predictive score or different lung functions showed no significant correlation to duration of IPPV, time of high oxygen concentration or high peak pressures (>20 cm H_2O)

DISCUSSION

Improved intensive care including assisted ventilation have resulted in increasing survival rates even for the smallest infants (17, 19, 27 30, 33, 38) In our clinic the use of *early* CPAP via the face chamber technique (3) has restricted the need for IPPV at HMD to the most immature infants and those arriving in a bad condition (20, 36) In AR, IPPV has to a great extent been replaced by other therapeutic measures such as low-pressure CPAP, refined adjustment of inspired oxygen and theophyllamine administration (20) In both HMD and AR, IPPV has thus been successively restricted to more immature infants Hence in the present material 50% of the patients had a birth weight of 1 2 kg or less and a gestational age of 29 weeks or less Selection of more immature patients often requiring prolonged IPPV, would increase the risks for pulmonary sequelae since the treatment may disturb the

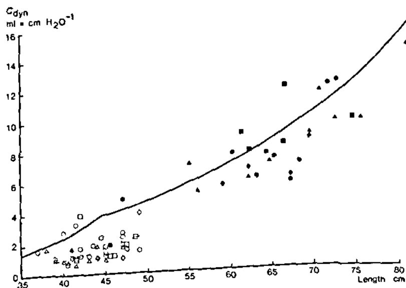


Fig 4 Dynamic compliance (C_{dyn}) in relation to length in 41 IPPV treated patients Symbols see Fig 3

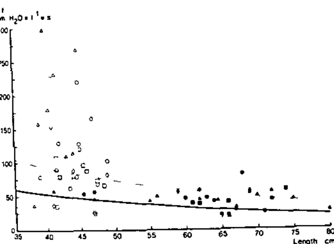


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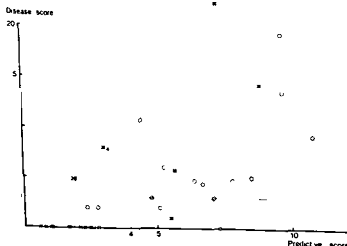


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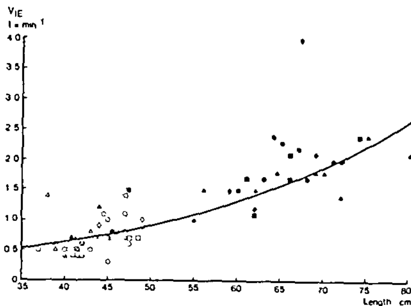


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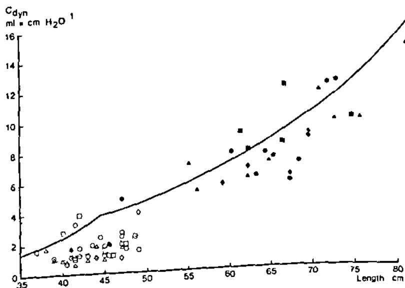


Fig 4 Dynamic compliance (C_{dyn}) in relation to length in 41 IPPV treated patients Symbols see Fig 3

reducing high oxygen concentrations when the patients improve during IPPV

BPD was the probable cause of death in one of 31 patients but none of the survivors in the present material showed progressive respiratory symptoms Instead there was a rapid improvement of lung functions during the first year After the first year pneumonia and bronchitis decreased in most infants This may be due to growing diameters of the small airways with less tendency of obstruction by mucosal swelling at airways infections

After 6 months of age X ray often showed overdistention even in patients with normal radiographs shortly after IPPV Overdistention was not correlated to duration of IPPV lung functions or lung diseases It has previously been reported in HMD survivors not treated with IPPV (26) We lack radiographic reference material for healthy 6–12 months old highly preterm infants without neonatal respiratory insufficiency Overdistention might represent an unspecific form of thorax and lung development in patients surviving very preterm births

Most follow up studies more than 5 years after assisted ventilation show a rapidly decreasing incidence of lung disease after the second year of life and only very few children with persisting respiratory disorders at the age of five (15 16 18 32) However Coates et al found signs of increased small airways resistance in 7 year old HMD patients treated with negative pressure ventilation (9)

In a study of a general population samples Burrow et al found a strong correlation between recurrent bronchitis in childhood and the prevalence of obstructive airway disease and ventilatory impairment with increasing age in adults (7) It is thus possible that IPPV treatment in the neonatal period represents an important risk factor for obstructive airway disease in adulthood To evaluate this it is necessary to continue follow up studies of IPPV survivours with lung function tests to adult age The children in the present study could form the base for such a study

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Table 5 *Duration (days) of IPPV, high inspired oxygen and high peak pressure in 19 HMD patients with BPD or normal chest X-ray (median and range)*

	Duration of IPPV	Inspired oxygen (%) ≥40	Peak pressure (cm H_2O) >20
BPD (*N* = 8)	32 (13–59)	2 (0–16)	2 (0–14)
Normal (*N* = 11)	15 (5–66)	5 (0 8–24)	3 (0–32)

Differences between groups not significant

lung disease than each examination used alone

As previously shown measurements of *f*, V_T and V_{tE} gave no information of lung function after IPPV (2) Since no patient had signs of upper airways obstruction the high values of R_f and R_{tE} probably indicate lesions of the small airways This is supported by morphologic studies that have shown obliterating bronchitis, bronchial muscular hypertrophy and peribronchiolar fibroblastic proliferation in IPPV treated HMD patients (5, 6, 24, 37) The decreased C_{dyn} shortly after IPPV in our patients agrees with previous studies (2, 8, 34) The strong tendency of normalization during the first year in all studies may reflect the rapid multiplication of air saccules that normally takes place in the last trimester followed by alveolization after 2 months of age in full-term infants (29, 35)

The cause or causes of lung damage are not clear Many authors have claimed that *oxygen toxicity* is the main etiologic factor (5 6, 24, 25, 31) Other reports have shown that high oxygen concentrations are not necessary for development of BPD (20, 28) Furthermore, although many patients with neonatal respiratory distress have been treated with high inspired oxygen alone or with CPAP very exceptional cases have developed BPD without IPPV (2, 10) In the present study neither radiographic changes nor later lung diseases correlated to time in high oxygen during IPPV

Immaturity per se has been proposed as etiologic factor (2, 10, 14, 20, 28) It has been suggested that the immature lung should be more vulnerable for oxygen toxicity owing to low levels of antioxidants such as superoxide dismutase and Vitamin E (4, 12) Taking lung maturation into consideration *barotrauma* may also be of importance At a gestational age of 30 weeks the gas exchanging air spaces comprises respiratory bronchioles and only one or two generations of saccules (29, 35) Taghizadeh &Reynolds showed that non surviving IPPV treated HMD patients who died during the first 2 weeks had an extensive overdistention of the terminal airways where as most saccules remained collapsed (37) In another study they showed that usage of lower peak pressures at IPPV reduced the incidence of BPD (30) Enhorning & Robertson found similar overdistention of terminal airways in surfactant deficient premature rabbits This was ascribed to lower surface forces in the terminal bronchioles than in the collabated saccules according to Laplace's law for cylindrical and spherical surfaces, respectively (11)

Thus IPPV treatment, especially with high peak pressures, may be one cause of damage to what will become the most peripheral air way The development of the last generations of saccules followed by alveolization could explain the rapid normalization of compliance The more longlasting increase of resistance could reflect a slower or less complete restoration of peripheral airway damage Such damage could also offer a reasonable explanation of lung disease of largely obstructive character after IPPV in this and other reports

In the present material no significant correlation between lung disease and duration of treatment with peak pressures over 20 cm H_2O could be found During the latter half of the study period, however, several HMD patients treated with IPPV for more than 2 weeks survived without BPD or later lung disease This could partly depend on our policy the last years of reducing high peak pressures before

Acta Pædiatr Scand 69 771–773, 1980

NOISE POLLUTION IN NEONATAL UNITS A POTENTIAL HEALTH HAZARD

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ABSTRACT Anagnostakis, D , Petmezakis, J , Messaritakis, J and Matsaniotis, N (First [illegible] University Athens, Greece) Noise pollution in neonatal [illegible] the noise level between normal nursery and NICU, [illegible] was recorded when the infant received supplemental oxygen, was under ventilator or when an air compressor was in operation High noise levels were equally the same both in a m and p m hours in the NICU As many high risk infants spend a long time in a NICU, there is an urgent need for further evaluation of noise levels and their effect on the outcome of infants

KEY WORDS Newborn infant, noise level neonatal unit

Recently much attention has been paid to the possible side effects of noise on human beings and especially on the newborn infants (2, 4, 9) The damaging effect of high intensity noise on the cochlea of human being is now well known (1) whereas there is some evidence that high noise levels stimulate the hypophyseal adreno cortical axis (11) and cause peripheral vaso constriction (13)

The subject is of particular interest to the neonatologist because it seems possible that there is an increased susceptibility to noise induced hearing loss in early infancy (7) and because noise levels in neonatal intensive care units are high enough due to the operation of noise producing devices Several studies have reported the ambient noise levels in infant in cubators (3 8 9 15) However very limited information exists about the additive effects of noise produced by the numerous mechanical devices operating in a neonatal unit (2)

The purpose of this paper was to measure the noise levels produced by the mechanical equipment used in an intensive care unit and to relate these levels to possible pathologic effects on the incubated newborn infant

METHODS

A Bruel and Kjaer precision sound level meter type 2203 was used to measure noise levels from the following sites

1 At the midpoint of the rooms of a neonatal intensive care unit (NICU) and of the normal nursery

2 Inside five electrically powered incubators from those in use in our NICU and more precisely

(*a*) at the midpoint and at the two sides of the mattress where the head of the baby is usually placed

(*b*) inside the incubator whenever the nurse opened the sleeves of the incubator

(*c*) under a plastic hood when an ordinary oxygen supply (5 l/min) was given

(*d*) under a plastic hood (as in *c*) and when supplemental oxygen was given through a humidifier

(*e*) beside the ear of the baby when the baby was under ventilator (baby bird)

(*f*) beside the ear of the baby when an air compressor was in operation

(*g*) inside the incubator when the infant was crying

Measurements from site 1 were made for four consecutive days every two hours throughout the day Thus at the midpoint of the NICU and of the normal nursery sound level was measured 48 times The number of measurements from all other sites are given in Table 1

All measurements were expressed as A weighed sound levels (dBA)

RESULTS

The noise levels measured in the normal nursery, the NICU and inside the incubator under the conditions described above are

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Submitted Febr 26 1980
Accepted May 12 1980

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respect it is perhaps of interest that at least in our NICU, noise levels were practically the same in both a m and p m hours, suggesting that high noise levels persisted throughout the day, a finding that was also reported from other NICU units (17). Recently Douek et al (6) presented clinical evidence further supporting the assumption that incubator noise may be responsible for hearing loss in low birth weight infants However, Abramovich et al (1) studying the hearing of 111 perinatal intensive care survivors at a mean age of 6 $^1/_2$ years found no evidence that ambient incubator noise had affected the hearing of their low birth weight infants

Other points of concern are the reported synergistic effect between ototoxic drugs and high noise on hearing loss of guinea pigs and the increased susceptibility to noise damage induced by these drugs (5, 10, 12)

It should also be remembered that except for hearing damage, excessive exposure to noise produces a variety of physiologic (stimulation of pituitary-adrenal axis, peripheral vasoconstriction) and psychologic disturbances (11, 13, 14)

In view of the findings of the present study and the studies of others it is recommended that (*a*) physicians and nurses should make a special attempt to limit unnecessary noise (excessive conversation, radios played at high volume in the NICU), (*b*) if an infant receives ototoxic drugs, noise levels of incubator and other noise producing devices should be regularly measured, (*c*) noise producing devices should be positioned as far from the newborn s head as possible and finally, (*d*) more research should be stimulated on the possible hazardous effect of noise in a baby suffering from hyperbilirubinaemia, hypoxia and/or septicaemia, conditions often encountered in low birth weight infants

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Submitted Febr 6 1980
Accepted May 14, 1980

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Table 1 *Mean (±1 S D) noise levels in the normal nursery the NICU room and inside the incubators*

Source	Noise level (dBA)	No of measurements
Normal nursery	44±1 6	48
NICU room	51±2 0	48
Inside the incubator		
At the middle of the mattress	53±0 9	20
At the sides of the mattress	55±1 0	20
Opening the sleeves of the incubator	63±0 8	20
Under the hood (with O_2 5 l/min)	70±1 8	15
Under the hood (with O_2 through humidifier)	75±1 5	15
Respirator in operation	65±1 7	10
Air compressor in operation	67±1 8	10
During baby s cry	75±85	10

shown in Table 1 There is a considerable difference in the noise level between nursery and NICU, this difference being as high as 6–8 dB (A) It is of interest that the simple opening of the sleeves of the incubator produces an additional noise of about 10 dB (A), whereas a considerable increase of noise is recorded when the infant receives supplementary oxygen and particularly through a humidifier Finally it should be pointed out that there is a difference in noise level of as much as 2–3 dB (A) depending on where in the incubator the measurements are done, i e at the middle or at each side of the incubator

The noise levels in the NICU recorded throughout two different days are shown in Fig 1 High noise levels were practically similar both in a m and p m hours It should be noted that the highest noise levels recorded at different hours each day corresponded to the admission of a high risk baby

DISCUSSION

In the present study the noise levels measured inside the incubator are in accordance with those founded in some recent studies (1, 8), but definitely lower than those reported a few years ago (3, 15) This obviously must be attributed to the efforts of manufacturers to reduce the noise from the machinery of the incubators In spite of this, noise levels inside the incubators were, in all instances, above 50 dBA, a level that has a 25% probability of seriously affecting sleep in adults (1) Although corresponding disturbing noise levels for neonates are not known, noise levels measured in this study show that the noise inside the incubator undoubtedly exceeds the level proved to be disturbing to the sleep of an adult

Perhaps even more important is the potentially hazardous effect of the noise to the hearing of the newborn infant Although the noise levels recorded in this study are below the damage risk criteria established for adults (16) it should be emphasized that the safe sound level for full term and even more for immature preterm babies are not known (2, 4) Furthermore, there is some evidence that at least in animal, infants may be more susceptible to noise-induced hearing loss than adults (5, 7)

In addition it should be pointed out that damage risk criteria for adults are based on intermittent exposure to noise, whereas newborns in incubators are exposed to continuous noise for periods of several days or weeks or even months with no recovery time (8) In this

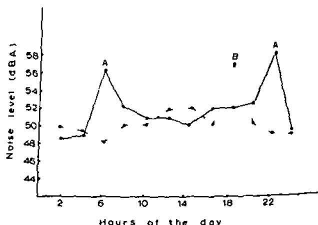


Fig 1 Noise level (dBA) recorded every two hours in the NICU room during two different days A (●—●) and B (● ●) The highest noise levels recorded (A A, and B) coincide with the admission of a high risk baby

LETTER TO THE EDITOR

Sir

Acta Pædiatrica Scandinavica, Volume 69, contained an article by Cavallo et al. entitled "Serum Levels of Thyrotropin, Thyroxine, 3 3',5—Triiodothyronine and 3 3',5 Triiodothyronine (Reverse T_3) in the First Six Days of Life" (pp. 43–47). In this study, serum was drawn from 140 term infants, irrespective of sex, and determinations made by radioimmunoassay.

We have recently published similar results for T_4 levels in infants (Tenore, Oberkotter & Koldovsky, *Early Human Development* 4 (1) 41–49), examining identical time periods in 240 term infants, and divided by sex (males, $n=120$, females $n=120$). Our results indicate highly significant sex differences between 12 and 48 hours wherein the maximum T_4 level attained in females during this period was found to be 18.3±3.3 μg/dl ($n=18$), but in males, 15.5±2.9 μg/dl ($n=21$) ($p>0.025$). If we ignore the sex of the infants, our data for this period are very much in agreement with those of Cavallo. Thus, it should be emphasized that the understanding of thyroid function in early postnatal life may require consideration of the infant's sex and suggests that other hormonal influences may be reflected in these functions.

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Acta Pædiatr Scand 69 777–778, 1980

LETTER TO THE EDITOR

Sir

Although some time has passed since the monograph ' Prevention of Rh Immunization in Finland ' (Supplement 274, 1978) was published, I think this letter still has relevance because in that study there was an important misinterpretation On page 23 is written Another retrospective study (1) failed to confirm Taylor s (2) results, but nevertheless RhIgG prevention was suggested for all Rh negative females born to Rh positive mothers"

This statement is not correct In the paper referred to it was clearly shown that there was a striking similarity in the percentage of newborn affected with erythroblastosis (*a*) between the 1st birth in the group where grandmothers were Rh positive (20%), and the 2nd birth in the group where grandmothers were Rh negative (20%), (*b*) between the 2nd birth in the group where grandmothers were Rh positive (38%), and the 3rd birth in the group where the grandmothers were Rh negative (30%) (*c*) and between the 3rd birth in the group where grandmothers were Rh positive (28%) and the 4th birth in the group where grandmothers were Rh negative (25%)

Thus Taylor s results were confirmed and it was concluded that an Rh positive grandmother acts similarly to the antigenic stimulation caused by pregnancy and birth of an Rh-positive fetus, and that logically it might be advisable to give anti D γ-globulin soon after birth to all Rh negative newborn females born to Rh positive mothers

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The Editor asked dr Eklund to comment on this letter

Sir,

As a comment on Dr Ramos de Almeida's letter, I would like, before looking at the data accumulated to prove the "grandmother" theory, to stress the fundamental importance of the fact that the normal ratio of Rh groups among mothers to Rh negative infants is 60% Rh positive, 40% Rh-negative Taylor, in a retrospective study, reported that an Rh-negative woman was significantly more likely to have a child affected with Rhesus HDN when the child's grandmother was Rh-positive However, there were 111 mothers with anti D and affected children and 41 of the grandmothers (36 9%) were Rh negative and 70 (63 1%) were Rh-positive, this ratio being insignificantly different from that expected Of 46 mothers who had had three or more Rh-positive ABO-compatible children without developing anti-D, 27 (59%) had Rh negative grandmothers and 19 (41%) Rh-positive grandmothers, i e , even a lower proportion of Rh-positive grandmothers than expected (3) In a subsequent retrospective study on the influence of a grandmother's Rh group on her Rh positive grandchildren, Ramos de Almeida & Rosado concluded "The data also show that Rh positive grandmothers do not cause in their families an excessive number of grandchildren affected by erythroblastosis This is in conflict

Acta Pædiatr Scand 69. 779–780, 1980

SHORT COMMUNICATION

THE DIFFERENTIAL LEUKOCYTE COUNT IN FULL-TERM NEWBORN INFANTS WITH MECONIUM ASPIRATION AND NEONATAL ASPHYXIA

The usefulness of the differential white blood cell (WBC) count in the diagnosis of infection in the newborn infant is now well established (1–3, 5, 7–10) Changes in the leukocyte count infants with the meconium aspiration ıdrome (MAS) and with neonatal asphyxia A) have been described (4) but the value the differential WBC count in these condiıns has not been investigated The present udy evaluates the changes in the differential BC count in MAS and NA in full-term innts and in establishing their value in deciding n treatment

One hundred and six newborn infants with IAS NA or both were studied prospectively or their differential WBC count Forty-six inants suffered from MAS, 47 from NA and 13 ıad NA with MAS All the infants were fullerm and had an appropriate weight for their gestational age Infants born to diabetic mothers, hypertensive mothers and mothers with fever were excluded Blood samples were taken within the first 36 hours of life as capillary blood from heel punctures Standard hematologic techniques were employed Standards for normal were derived from the assessment of 100 separate WBC counts obtained from normal full-term infants between 12 to 24 hours of age According to our laboratory we consider a value of over 2000/mm^3 of immature neutrophil cells (band forms) and a ratio of bands to total neutrophil cells (B/N ratio) equal or over 0 14 for the first leukocyte count to be pathologic. None of these infants received antibiotics and a complete sepsis work-up done in all the newborns with abnormal leukocyte counts revealed no positive blood, CSF or urine cultures A second WBC count was performed in all the infants after 96 hours of life.

The results of our study are presented in Table 1 Twenty-six % of MAS group had an elevated absolute number of bands or an abnormal B/N ratio Overall, 32 6% of infants with MAS had at least one abnormality in their initial differential leukocyte count and in 19 5% both criteria were pathologic The difference between the absolute bands and the B/N ratio in the newborns with MAS and in the control group was very significant (p<0 001) The newborns with NA had absolute numbers of bands elevated in 15 cases (31 9%) and the B/N ratio was abnormal in 17 cases (36%) Overall, 42 6% of newborns with NA had at least one hematological abnormality and 25 5% had both criteria within

Table 1 *Number of infants with increased bands and abnormal B/N ratio*

Group	Total number of cases	Absolute number of bands/mm^3 >2000	B/N ratio >0 14	Both criteria*
Meconium aspiration	46	12	12	9
Neonatal asphyxia	47	15	17	12
Meconium aspiration with neonatal asphyxia	13	3	4	2

bers of bands + B/N ratio

with Taylor's results which showed an increased number of mothers with affected children in families with Rh-positive grandmothers" (2) This in fact is a misinterpretation because Taylor's group of immunized mothers was the one showing normal Rh patterns It is obvious that if the "grandmother' theory were true, there should be a greater proportion of Rh positive grandmothers than expected. Our data show a ratio of Rh-positive/Rh-negative grandmothers, which is exactly that expected based on calculations of gene frequencies in a Finnish population (1) Intrapartum sensitization of Rh negative infants to the D antigen must therefore be rare and routine administration of RhIgG to Rh-negative female infants is therefore not indicated

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Acta Pædiatr Scand 69 781–782, 1980

SHORT COMMUNICATION

CORTICAL BLINDNESS IN A CHILD WITH ACUTE LEUKEMIA[1]

Ophthalmic complications are detected in about 9% of all children suffering from acute leukemia (1) The typical complications are retinal bleeding and leukemic infiltration of the optic nerve, retina, iris or orbit Loss of sight due to optic nerve infiltration was seen in 9 of 657 cases Two cases of peripheral blindness, believed to be a side effect of combined modality therapy have been reported (2) However, cortical blindness has not been described Although it is believed that cortical blindness is rare in patients with leukemia, thanks to the blood–brain barrier, we have experienced an unusual case of acute promyelocytic leukemia (APL) in which cortical blindness developed

Case report An 18-month old baby girl with exophthalmus (l>r) a retrobular tumor and an abnormal blood picture was admitted to this hospital on Dec 24 1976 She had a large head (circumference 52 5 cm >+2 S D) and an enlarged frontal fontanel (5×5 cm) with bulging Swelling of soft tissue like a rachitic rosary was found in costosternal junctions on both sides of the chest Complete blood counts showed hypochromic anemia Bone marrow smears showed 2×10^4 nucleated cells mm^3 and 68 8% promyelocytes The patient was diagnosed as having APL (3) and chemotherapy was started Complete remission was rapid and the retrobulbar tumor disappeared The head circumference also decreased to 50 cm Three months later the patient suffered a hematological relapse and a second remission was induced by POMP therapy (4) Meningeal leukemia occurred in the fourth month but a favorable response was obtained by intrathecal administration of cytosine arabinoside (ITCA) After that temporary visual disturbance left hemiplegia and extensive subdural hematoma were observed These symptoms were improved only by subdural tapping

Thirteen months after diagnosis the patient again developed left hemiplegia and bilateral loss of vision and became somnolent Cerebrospinal fluid assay and ophthalmoscopic examination failed to reveal any abnormalities These finding suggested that her blindness was of cortical origin She was treated with cranial irradiation with 2000 rad but her visual acuity was not restored However she regained consciousness and the left hemiplegia gradually decreased An EEG taken on March 2 1978 showed a flat response to photic stimulation Six months after she became blind the patient developed intraventricular bleeding and died

Cortical blindness was confirmed at autopsy Atrophy of the visual cortex and necrosis of white matter were seen macroscopically No significant change was noted in the chiasm or optic nerves Microscopic findings showed leukemic infiltration into the subarachnoid space around the visual cortex In the cortex (including the visual cortex), extensive degeneration of neurons and gliosis were found Necrosis of the white matter was also marked, sometimes presenting a cavernous appearance

The visual loss caused by leukemic infiltration was assumed to be of acute emergency and was taken as an indication for radiotherapy (1) In our case, the whole brain was irradiated with 2000 rad immediately after the patient became blind, but it did not restore the vision Thus this cortical blindness caused by leukemic infiltration did not respond to radiation therapy, unlike peripheral blindness The patient developed temporary visual disturbance first This is considered to have been caused mainly by anoxia, not by infiltration, because extensive subdural hemorrhage was observed over the visual cortex by computed tomography (5, 6) In fact, this symptom was reversed only by subdural tapping

Bone changes in leukemia have been observed in about half the cases studied by X-ray examination, and these changes are generally characterized by (i) diffuse demineralization, (ii) the appearance of metaphyseal bands, (iii) osteolytic lesions, (iv) a periosteal reaction, (v) osteosclerotic lesions, and (vi) mixed lesions (7)

On the other hand, X-ray examination of our patient revealed rachitic change of the ribs These changes waxed and waned parallel

[1] Presented in part at the Fourth Meeting of the Asian Pacific Division of the International Society of Hematology, June 26 1979 Seoul South Korea

the pathological range The difference between the NA group and the control group was significant ($p<0.05$) for bands and very significant ($p<0.001$) for the B/N ratio In the small group of infants with MAS and NA together we obtained almost the same results—38 4% of all cases with at least one hematological abnormality and in 15 3% both criteria were abnormal In all the newborns studied the bands' count was between 400–600/mm³ (normal range) after 96 hours of life Repeated platelet counts were also within the normal limits

The present study has demonstrated that in infants with MAS and/or NA the presence of elevated band forms and/or abnormal B/N ratio is a common finding In all the newborns these hematological changes were transitory and the differential count returned to normal within 4 days We speculate that in neonates with MAS and/or NA the leukocyte abnormalities express the infant's reaction to the stress and hypoxic stimulation produced by these conditions (6) This hematological picture is very similar with that seen in neonatal infections Thus, the differential leukocyte count is of much less value in these two groups of infants and other criteria will have to be used in the diagnosis of infection If there are perinatal risk factors (maternal fever, prolonged rupture of membranes etc) and/or clinical and radiological signs, we prefer to treat with antibiotics until the results of cultures are obtained In the absence of these factors, we place the infant in an incubator for close observation and do not treat with antibiotics

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Acta Pædiatr Scand 69 783–785, 1980

CASE REPORT

INTERRUPTED AORTIC ARCH IN TWO SIBLINGS

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AB [illegible]
La [illegible]
Ra [illegible]
In [illegible]
with identical malformations consisting of complete interruption of the aortic arch [illegible]
[illegible] septal defect patent ductus arteriosus and anomalous origin of the right sub-
[illegible]

KEY WORDS Interrupted aortic arch, identical cardiac malformations in siblings, genetic counselling

Complete interruption of the aortic arch is a rare congenital malformation, the highest incidence reported being 1 4% in an autopsy material (1) Hitherto only one report has been published about this malformation in siblings (2) We present here the findings in one further pair of siblings as this report together with future reports may contribute to the understanding of the genetics

CASE STUDIES

Case 1

This girl was the first child of a healthy mother There were no known cardiac malformations in the family history The mother had had no diseases during the pregnancy and she had taken no medication The pregnancy and delivery were uncomplicated She was born at term with a birth weight of 2800 g At the age of 4 days she developed heart failure and on admission she was in severe distress with acidosis She had no facial abnormalities Serum calcium was 2 59 mmol/l (normal 2 3–2 7 mmol/l) Heart catheterization with angiocardiography revealed complete interruption of the aortic arch between the left carotid artery and the left subclavian artery (type B) The right subclavian artery arose from the descending aorta The ascending aorta was considerably smaller than the pulmonary artery There was a concomitant ventricular septal defect with a large left to right shunt a patent ductus arteriosus and an open foramen ovale

She died on the following day and the autopsy confirmed the catheterization findings No other malformations were demonstrated in particular the thymus was found to be normal The parathyroid glands were not searched for

Case 2

This girl was admitted one year after the first case She was the only and younger sister of case 1 The mother had had no diseases during the pregnancy and had taken no medications The pregnancy and delivery were uncomplicated She was born at term with a birth weight of 3000 g She had no facial abnormalities Serum calcium was not recorded At the age of 4 days she too developed heart failure Two days later heart catheterization and angiocardiography revealed identical malformations to those found in her sister She died on the following day and autopsy confirmed the diagnosis No other malformations were demonstrated the thymus was normal and the parathyroid glands were not searched for

DISCUSSION

One report only of siblings with interruption of the aortic arch has been published (2) In their report the first child was male and the

with changes in the peripheral blood These manifestations are very rare, the only previous report we know of is a case of chloroma in a 10 month old girl reported by Austin et al (8)

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Submitted March 24 1980
Accepted June 10 1980

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second female Both had an interruption of type B (3) as in the case of our two sisters presented here, but both with an additional subvalvular aortic stenosis Levin et al (4) reported a pair of monozygotic twins, in one of whom a type B interruption of the aortic arch was found, while the other had a coarctation and hypoplasia of the aortic arch All cases had a patent ductus arteriosus and a ventricular septal defect Also in these reports there were no known diseases or medications during the pregnancies, which could account for the malformations The possible genetic background is still unknown Nora (5) believes that the genetic contribution to the etiology of congenital heart disease is through a multifactorial inheritance system in which a predisposition to the development of a specific congenital cardiac anomaly is effected by selected environmental influences Since the disease bears some relation to preductal coarctation, it might be justified to compare it with the genetics of this disease In coarctation there is a slight tendency to familial occurrence (6, 7, 8, 9) The observed risk of recurrence is in the range of 1/212 (6) to 5/281 (8) If the multifactorial inheritance model of Nora is accepted, a rare disease carries a smaller risk of recurrence than does a common disease The recurrence risk in first degree relatives is higher for parents and children than for siblings (8)

There are very few clues to the possible environmental factors involved in the development of this entity Anticonvulsants may be involved in some cases (8) Some authors have suggested that hypoplasia of the aortic arch may be secondary to a decreased fetal aortic flow, due to a large intracardiac shunt (1 10) Wilson & Warkany (11) have described frequent anomalies of the aortic arch often in combination with ventricular septal defect in the offspring of vitamin A deficient rats Harris & Nyheim (12) have described idiopathic hypercalcemia of infancy with interruption of the aortic arch The possible causal effect of this remains to be proven

Interrupted aortic arch is reported in patients with DiGeorge syndrome, a familial and developmental anomaly of hypoparathyroidism, thymic aplasia and facial abnormalities Finley et al (13) have reviewed the literature and found interrupted aortic arch in 10 of the 54 reported cases The patients presented here apparently did not have DiGeorge syndrome

In the 8 year period from 1971 to 1979, we have diagnosed interrupted aortic arch in a total of 7 patients, including the siblings presented here None of the parents had any heart disease These patients had 6 brothers and sisters, of whom one was operated for coarctation of the aorta, while 5 were normal One of the mothers received primidone for epilepsy and one had infectious mononucleosis in the first trimester No other possible etiological factors could be demonstrated

The present report underlines the partly genetic etiology for interrupted aortic arch The potential risk for familial occurrence cannot be calculated at present A more firm basis for genetic counselling can only be obtained when several centres are reporting their experiences

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Acta Pædiatr Scand 69 787–792, 1980

CASE REPORT

UNUSUAL CLINICAL AND ULTRASTRUCTURAL FEATURES IN A BOY WITH BIOCHEMICALLY TYPICAL MANNOSIDOSIS

B A GORDON R CARSON, and M DARIA HAUST

From the Departments of Biochemistry Paediatrics and Pathology the Children s Psychiatric Research Institute and the University of Western Ontario London, Ontario Canada

ABSTRACT Gordon, B A., Carson, R and Haust, M. Daria (Departments of Biochemistry, Paediatrics and Pathology, the Children's Psychiatric Research Institute, and the University of Western Ontario, London, Ontario, Canada) *Unusual clinical and ultrastructural features in a boy with biochemically typical mannosidosis* Acta Paediatr Scand, 69: 787, 1980.—A 4½ year old boy with a history of recurring respiratory tract infections and seizures, and evidence of severe retardation of psychomotor development and growth, lacked the coarse facial features, skeletal changes and other clinical stigmata generally associated with mannosidosis, but the total α-mannosidase activity in his leukocytes, cultured fibroblasts and liver were no more than 10 % of the control mean Studies of the residual α mannosidase enzyme suggest a specific deficiency of the thermostable isoenzyme with an acidic pH optimum. The α-mannosidase in the fibroblasts of our and another (control) patient with mannosidosis had a reduced affinity for the substrate 4-methylumbelliferyl-α D-mannoside Light microscopy of the liver biopsy showed an increase in connective tissue often distorting the hepatic

those described in reported mannosidosis patients, and in addition several forms of secondary lysosomes, prominent peroxisomes (microbodies), increased numbers of profiles of smooth endoplasmic reticulum, dilated rough endoplasmic reticulum containing traces of fine granulo-fibrillar material, increased numbers of rosettes of α particles of glycogen and reduced numbers of mitochondria with alterations in their distribution, size and configuration It is believed that the usual clinical and hepatic ultrastructural features in our patient reflect another variant of mannosidosis

KEY WORDS Mannosidosis, enzyme activity, liver, dermal fibroblasts, tissue acid hydrolases

Mannosidosis a lysosomal storage disease, results from defective glycoprotein catabolism Since the initial report by Ockerman in 1967 (15) over 30 patients ranging in age from a few months to 26 years have been described (17) Recently we have investigated a boy who showed only a few physical characteristics of mannosidosis, whereas the biochemical data and some morphological features of the liver were in keeping with that disease However, other hepatic alterations were at variance with those reported previously

The purpose of this short communication is to draw the attent on to the probability that mannosidosis may be expressing itself in a broader spectrum of clinical and structural manifestations than has been appreciated to date

CLINICAL AND LABORATORY DATA

The male patient (CW) was the second child of unrelated Irish parents The pregnancy was normal with the excep-

Supported by grant in aid 712 from the Ontario Mental Health Foundation Toronto Ontario Canada

Presented in part at the Fifty Second Annual meeting of the Canadian Paediatric Society, June 9 1975 Toronto, Ontario, Canada (9)

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Table 1 α-Mannosidase activity

M 4 methyl
in a final
pH 4 0 con
taining 20 μl of the enzyme source After incubation at 37°C for 30 min the reaction was stopped by the addition of 3 ml of 0 2 M glycine buffer pH 10 7 and the fluorescence measured against a 4 methylumbelliferone standard in an Aminco Bowman spectrophotofluorimeter with an excitation wavelength of 365 mμ and an emission wavelength of 450 mμ

	CW	Control	
		Mean	Range
Serum[a]	7 8	21 8	13 7–34 3
Leukocyte[b]	1 5	85 1	48 0–151 1
Dermal fibroblasts[b]	12 2	116 1	75 6–173 7
Liver[b]	2 31	25 3	17 4–33 0

[a] mμM/ml/h
[b] mμM/mg prot/h

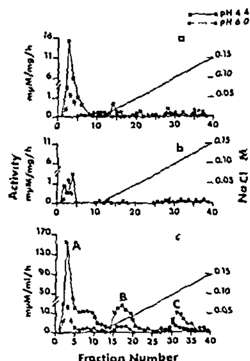


Fig 1 Elution profile from a DEAE cellulose column at pH 6 0 of supernatant from homogenates of dermal fibroblasts of NC patient (a) and patient CW (b) as well as of NC liver (c) The α mannosidase activity was assayed at pH 4 4 and 6 0

tion of a mild toxemia in the third trimester The labour and delivery were uneventful and at birth the infant weighed 6 lb 10½ oz The Apgar score at 1 and 5 min was 9 The infant was discharged in good health at 5 days of age At home he became increasingly irritable was difficult to feed cried incessantly and at 5 months a paediatrician diagnosed cerebral palsy of unspecified etiology The head circumference was below the 3rd percentile and his weight and length were at the 10th percentile The Moro tonic neck and grasp reflexes persisted There was little head control and the peripheral reflexes were exaggerated The infant smiled at 3 months but further development was negligible At 8 months he had the first febrile convulsion which was followed by repeated fever related grand mal seizures Before 4½ years of age he was admitted to hospital nine times for treatment of bronchitis and pneumonia and twice for the evaluation of seizures At this age he was referred to Children s Psychiatric Research Institute (CPRI) On admission he was emaciated mentally retarded and quadriplegic with spasticity of all muscle groups There was no spontaneous motor activity and he lay in an opisthotonic position with his legs splayed out in a frog like posture unable to roll over or sit with support There were mild flexion contractures of his wrists elbows ankles and knees The peripheral reflexes were exaggerated the plantars were upgoing and there was bilateral ankle clonus He did not speak and had a constant irritable whine Audiological examination revealed adequate hearing (for speech) Psychometric assessment placed him in the range of profound mental retardation His weight (10 5 kg) length (96 cm) and head circumference (46 5 cm) were all below the 3rd percentile for his age The facial features were normal and not reminiscent of those in mucopolysaccharidoses There was a slight internal strabismus of the lef ... lobe of
bo

the antibody titres to toxoplasma rubella cytomegalovirus and herpes were all within normal limits In serum the activities of α fucosidase and β galactosidase were normal while β N acetylhexosaminidase and β glucuronidase were increased 3 and 4-fold respectively The activities of the above acid hydrolases and aryl sulfatase A were normal in leukocytes No dysmorphic bony changes were revealed radiologically There was evidence of an iron deficiency anaemia Observations made on the peripheral leukocytes and their bone marrow precursors are reported elsewhere (8)

BIOCHEMICAL STUDIES

Materials and methods Activity of α mannosidase was determined in the serum leukocytes cultured dermal fibroblasts and hepatic tissue of the patient as indicated in Table 1 Serum and leukocytes from 7 healthy children and adults dermal fibroblasts from 4 subjects with inherited metabolic or chromosomal anomalies and normal liver obtained at necropsies of 3 age matched subjects were utilized as normal controls (NC) Dermal fibroblasts cultured from a patient with documented mannosidosis (courtesy of Dr J W Callahan) served as an abnormal control (AC) The harvested cultured fibroblasts were homogenized in 2 ml of distilled water the homogenate was centrifuged and the supernatant used in the enzyme assay The supernatant obtained by centrifuging the homogenate of patient s liver (12 1 mg in 2 4 ml of distilled water) at 600 × g for 10 min served for the assessment of hepatic enzymes



Fig 2 There are hepatocellular atrophy broad sinusoids and increased perisinusoidal connective tissues which often surround single mono- and binuclear (arrow) hepatocytes Small dark bodies and vacuoles are present in many hepatocytes Plastic-embedded Toluidine blue stain ×900

Fig 3 Some dilated profiles of endoplasmic reticulum in the hepatocyte appear to abut or be in continuity with the vacuoles (arrows) and occasionally contain a few membranous arrays (double arrow) Bulbous and constricted segments, and lateral 'budding' are evident in some mitochondria (m) Electron micrograph ×10000

The α mannosidase isoenzymes from cultured dermal fibroblasts of the patient CW and NC were compared with those in a normal liver All enzymes were chromatographed on a 0 9 × 15 cm DEAE cellulose column as described for the leukocytic enzyme (14) Thermostability studies on the fibroblast α mannosidase were carried out by the method of Beaudet & Nichols (4)

Results The α mannosidase activity in the serum of CW was 36% in leukocytes less than 2% and in the fibroblasts 10% of the mean values of the respective NC In the liver the activity at pH 4 0 was 9% (Table 1) but when assayed at pH 5 5 it increased to 17% of the NC level The level of β-galactosidase activity in the CW liver was twice that of the NC hepatic tissues "Resolution of the isoenzymes of fibroblasts from NC by chromatography on DEAE cellulose revealed a predominantly acidic form which was not retained on the column and correspond to the A isoenzyme (Fig 1) and a small delayed acidic peak which corresponded to the B peaks of NC liver The residual enzyme from the CW-fibroblasts was not retained by the ion exchanger However unlike the major component in the NC cells it appeared to consist of an approximately 1 1 mixture of the acidic and neutral species

Thermostability studies of the α mannosidase from NC fibroblasts showed an activity reduced only to 94% of the starting level when preincubated at 53°C for 15 min, whereas the activity in fibroblasts from CW and AC was

relatively thermolabile and was reduced to 25% and 22% respectively

In the studies on the relation between the substrate (4-methylumbelliferyl α D mannosidase) concentration and the α mannosidase activity assayed at pH 4 0 it was observed that the Km of the enzyme in extracts from fibroblasts of two NC was 0 9 and 1 2 mmolar while that from CW was 25 and from AC was 50 mmolar

MORPHOLOGICAL STUDIES

Materials and methods A portion of the percutaneous needle biopsy of the liver of CW as well as hepatic tissue from 3 age matched NC were processed for electron microscopy by standard methods (10) Sections of plastic embedded tissue were stained with toluidine blue for light microscopic survey Thin sections of areas selected were stained doubly and examined in an electron microscope

Results By light microscopy (Fig 2) some areas appeared normal but changes involving the architecture and hepatic cells were also present The small size of the sample precluded the assessment of the lobular pattern and periportal tracts There was widespread albeit subtle loss of parenchyma as indicated by the prominent sinusoids and increased collagen the latter surrounded single hepatocytes and impinged upon the spaces of Disse Atrophy of parenchyma often was accompanied by the presence of enlarged or binucleated cells The hepatocytes contained usually small cytoplasmic vacuoles tiny lipid droplets and round dark or pale bodies The prominent sinusoidal spaces were actually narrowed by proliferating perisinusoidal connective tissues and by protrusion of lining cells which were distended with droplets or vacuoles and often showed erythrophagocytosis Sinusoidal spaces contained cellular debris representing partially sloughed off or entirely disintegrated hepatocytes

Electron microscopy (Fig 3) confirmed the small size of hepatocytic vacuoles these had a diameter similar to but seldom larger than thrice that of a mitochondrion They varied in number from cell to cell (usually not exceeding one dozen) were bound by a unit membrane (often fading into the surrounding cytoplasm) occasionally fused with each other and most contained a homogenous slightly electron opaque or granular and finely flocculo filamentous substance and a few membranous arrays The cytoplasm also contained ovoid spherical or slightly angular organelles which measured 0 3–0 5 micron in diameter and had features characteristic of peroxisomes numerous profiles of smooth (SER) and rough (RER) surfaced endoplasmic reticulum and rosettes or 900 Å alpha particles of glycogen Many profiles of SER and RER were dilated and some contained traces of finely floccular or filamentous substance and a few membranous arrays Occasionally the vacuoles and dilated RER abutted upon or fused with each other and other cellular components particularly with the peroxisomes and the peribiliary dense bodies Golgi zone derived vesicles were numerous particularly at the microvillous surface facing the spaces of Disse The mitochondria appeared abnormal with respect to their size and shape Some were unusually long and/or branching consisted of alternating narrowed and bulbous segments and showed lateral bulbous portions However their internal structure was not altered nor was the diameter increased to that of the bizarre giant forms known in some other metabolic disorders (10 12) The changes observed in the sinusoids their lining and the adjacent spaces of Disse closely paralleled those seen on light microscopic examination In addition conspicuous bundles of collagen were present in the sinusoidal recesses and the cellular surfaces of adjacent hepatocytes showed prominent microvillous interdigitations which extended over a considerable length

DISCUSSION

The biochemical data pertaining to our patient (CW) were in keeping with those reported for mannosidosis (17) Thus the total mannosidase activity in the liver, leukocytes serum and cultured dermal fibroblasts was diminished The mannosidase isoenzymes from normal fibroblasts when separated on a DEAE cellulose column showed A and B isoenzymes but in the patient cells only the A isoenzyme remained Moreover unlike the normal A isoenzyme that was 15 fold more active at pH 4 4 than at 6 0 the residual activity in the CW fibroblasts was similar at both pH values In keeping with the previously reported data on thermal inactivation or pH dependence (3 4 7 14) there was a deficiency of the thermostable acidic mannosidase from the CW (and AC) fibroblasts and a reduced affinity of the residual α mannosidase for its substrate in the fibroblasts from both CW and AC

Some clinical features differed however from those reported previously (15 17) The coarse facies skeletal changes corneal or lenticular opacities visceromegaly and hearing defects were absent in CW but the patient did have recurrent respiratory tract infections

The structural alterations of the liver were also at variance with those reported previously These were described as enlarged hepatocytes with multiple clear Periodic Acid Schiff (PAS) negative vacuoles (5) swollen reticuloendothelial cells but no convincing storage phenomena (13) and only slight alterations consisting of some accumulation of

lymphocytes around portal tracts, small, PAS, and alcian blue negative cytoplasmic vacuoles normal sinusoidal cells and no fibroblasts (2) Our light microscopic examination showed a slight but definite increase of connective tissues in the perisinusoidal spaces of Disse which also encircled and isolated individual hepatocytes These cells contained numerous vacuoles small dense bodies and lipid droplets The sinusoidal cells also contained vacuoles and exhibited erythrophagocytosis Large pools of hepatoxytic debris at times almost filled the sinusoidal spaces

Our observations at the ultrastructural level differed considerably from those reported previously Ormas et al (16) who first described the hepatocytic vacuoles in mannosidosis believed these to be similar to those of genetic mucopolysaccharidoses Tubular structures myelin figures and electron dense aggregates (2) or stacks of fine fibrils (6) were reported to occur in the vacuoles in subsequent publications (2 5 6) in addition to the reticulogranular substance (16) The vacuoles varied from 1 5 to 9 0 μm and those in sinusoidal cells and bile duct epithelium from 0 3 μm to several μm (2) Paucity of peribiliary dense bodies and remnants of vacuolated tissues within sinusoids were observed in two patients (2) In all accounts it was mentioned explicitly that glycogen mitochondria and other cytoplasmic organelles appeared normal (2 5 6) However in our patient the hepatocytes showed in addition to the vacuoles increased amounts of rosettes or alpha particles of glycogen often in close association with the markedly increased profiles of SER dilated cisternae of RER containing finely granulo-filamentous substance a considerable number of peroxisomes (microbodies) and a variety of secondary lysosomes The hepatic vacuoles were considerably smaller and fewer in our patient than those reported previously and whereas the descriptions of their contents implied a similarity to vacuolar substances in genetic mucopolysaccharidoses this may have been only apparent Judging from the figures provided in the previous reports, as well as from observations in our case, the hepatic vacuoles in mannosidosis contain only traces of the above various substances In the present study the vacuoles were neither as large nor as numerous as those observed in genetic mucopolysaccharidoses (9, 12) The origin and nature of the vacuoles are uncertain, their presumptive lysosomal nature (5 6) is at best speculative The possible origin of vacuoles from the enlarging cisternae of SER or RER is supported in our observations by images showing stages transitional between these and cisternae, and fusion with the latter, and contents that are similar in SER or RER, and in the vacuoles

It is not understood just how the abnormally shaped mitochondria are the result of or participate in the above overall process Hepatic mitochondrial forms reminiscent of, but not identical to, those observed in our patient were described in a patient with hyperornithinemia and gyrate atrophy of the choroid and retina (1)

Considering all factors, it is reasonable to assume that the structural hepatic changes as well as the clinical features in the presently reported patient are a reflection of yet another true (genetic) variant of mannosidosis Alternatively it may be postulated that the disease under consideration expresses itself in a wide gamut of phenotypical and structural manifestations

ACKNOWLEDGEMENTS

The authors wish to thank Mrs Vera Feleki Miss Theresa Kwasowska M Irena Wojewodska Mrs Renate Feulgen and Mr Roger Dewar for their skillful technical assistance and Mrs Marion Webster for efficient typing of the manuscript

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Accepted April 19, 1980

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Acta Pædiatr Scand 69 793–796 1980

CASE REPORT

DIALYSIS ENCEPHALOPATHY IN A NON DIALYSED URAEMIC BOY TREATED WITH ALUMINIUM HYDROXIDE ORALLY

E NATHAN and S E PEDERSEN

From the Department of Paed atrics Kold ng Hospital Kolding Denmark

ABSTRACT Nathan, E and Pedersen S E (Department of Paediatrics, Kolding Hospital, Kolding Denmark) Dialysis encephalopathy in a non-dialysed uraemic boy treated with aluminium hydroxic [illegible] a P d atr Scand 69 793, 1980.—Brain aluminium concentration has been [illegible] than in uraemic pa [illegible] in haemodialysed patients We report a [illegible] uraemic boy showing symptoms of severe encephalopathy He was never dialysed but only treated with aluminium hydroxide orally Baluarte reported corresponding symptoms in non-dialysed uraemic children, but brain aluminium concentrations were not reported His patients as well as ours had very high levels of parathormone which may play a role in the resorption and distribution of aluminium Aluminium preparations should be avoided in children with renal failure

KEY WORDS Aluminium, dialysis encephalopathy, uraemia hyperparathyroidism

In 1972 Alfrey et al (1) described a progressive neurological syndrome which was later named dialysis encephalopathy syndrome (DES) Since then this syndrome has been recognized by many others (5 11)

It has been shown that the contents of grey matter aluminium in brain are very high in patients suffering from this syndrome (2 9 15) In patients maintaining haemodialysis a correlation has been reported between the aluminium concentration in the dialysate and DES (8 9 20) In adults the syndrome has only been described in haemodialysed patients Baluarte et al (4) observed symptoms which were clinically similar to DES in 5 children with chronic renal failure treated with aluminium hydroxide They had not been haemodialysed The concentration of aluminium in the brain was not measured

We report a case of high concentration of aluminium in the brain of a uraemic boy showing the symptoms of DES He had not been dialysed but only treated orally with aluminium hydroxide

CASE REPORT

The boy was referred to the paediatric department Kolding Hosp tal at the age of 6 months due to uraemia caused by bilateral congenital renal hypoplasia His osteodystrophy was treated with vitamin D and later with 1 alfa hydroxycholecalciferol At the age of 2 years treatment was started with oral ingestion of aluminium hydroxide to control his hyperphosphataemia An average of 43 mg aluminium/kg/day or a total of about 960 g was given to the age of 6 years He then had attacks of visual disturbances headache and very short drop seizures After a month he developed frequent general zed myoclonic jerky movements head drops and several grand mal seizures resistant to antiepileptic treatment He had asterixis fibrillations ataxia with exceedingly poor balance and co-ordination and his speech became slow deliberate incoherent and scanning with a stuttering quality

He also developed severe impairment of memory loss of abil ty to concentrate and his mental development stopped He became progressively confused and his consciousness was dimmed His symptoms were fluctuating but progressive until he was lying unconscious without any seizures He d ed 8½ years old

The creatinine clearance was 15 ml/min/1 73 m² surface area until he was 6 years old At 7 years old when his condition was rapidly deteriorating the creatinine clearance had fallen to 9 ml/min/1 73 m² and not until shortly before his death was it about 5 ml/min/1 73 m² No signs were found of tumor or virus infection Plasma calcium was mostly at the upper range of normal The concentra

tion of parathyroid hormone was increased with a maximum value of 13 times the upper normal limit Eight years old plasma magnesium was 1 62 mmol/l The symptoms did not change when it was normalized

The EEG initially showed innumerable paroxysmal diffuse rhythmical bursts of high voltage delta activity and occasional spike components together with delta and teta activity mixed with the ground pattern (Fig 1) Late in the course there were no paroxysmal bursts but only pronounced generalized delta and teta activity (Fig 2)

At autopsy bilateral renal hypoplasia and a moderate pancreatitis were found The brain showed chronic non specific degenerations and several microscopic calcifications The concentration of aluminium in the brain was determined with flameless atomic absorption spectrometry as described by McDermott et al (15) The level of aluminium in the grey matter was 80 0 μg/g dry weight and in the white matter 47 μg/g dry weight

Fig 1 Representative segment of EEG at the start of symptoms

DISCUSSION

The correlation between high aluminium concentrations in the brain and DES seems well established (2, 9, 15) The progressing encephalopathy of our patient was in accordance with that of the dialysis encephalopathy syndrome The EEG findings, which can be found before the symptoms appear (5) were present also in our patient The concentration of aluminium in the brain and the proportion between aluminium in grey and white matter was in accordance with the findings in patients suffering from DES (Table I)

The symptoms started after 4 years treatment with oral aluminium hydroxide At that time the total consumption was about 960 g aluminium or an average of 43 mg/kg/day The dose was then reduced and at his death the total intake was about 1 200 g aluminium

A high aluminium concentration in plasma is found in all patients treated with haemodialysis also in those without the encephalopathy

Table I *Aluminium concentrations as found in dialysis encephalopathy syndrome and in this case*
Values are the reported mean ± S E M mikrog/g dry weight

Category	No of patients	Grey matter	White matter	Mixed
Normal (2)	5	2 18+ 0 69	2 00+0 63	1 30± 0 68
Dialysed without DES (15)	12	5 1 + 2 8	3 5 +2 4	4 8 + 2 8
DES (15)	7	20 4 ±16 7	6 9 +5 3	16 0 ±11 9
DES (2)	7	24 98± 9 10	5 59±1 88	8 91± 4 29
DES (9)	6			66 1 (36 0–96 3)
This patient	1	80 0	47 0	

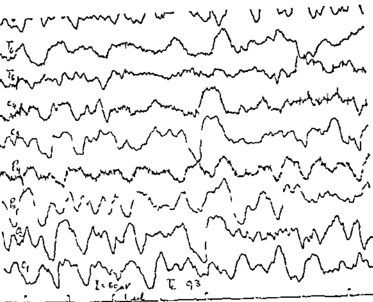


Fig 2 Representative segment of EEG late in the course (The changes were present in all leads)

(10) Aluminium is concentrated in the bones of patients maintaining haemodialysis and treatment with oral aluminium, yet the significance of this has so far not been established (17) Some bone symptoms and X ray findings however may be caused by aluminium (16)

Crapper et al (7) found elevated aluminium in brains from patients suffering from Alzheimers disease This was not in accordance with the findings of McDermott et al (14), who found the alterations correlated to age and not to the disease

Recovery from DES is uncommon It has been described following peritoneal dialysis (12) subtotal parathyroidectomy (3) and interruption of haemodialysis and aluminium intake (18) Even with very low concentrations of dialysate aluminium little if any aluminium appears to be removed from plasma by dialysis (10)

Balance studies have shown that adults with renal failure ingesting 1½–3 g of aluminium hydroxide absorbed 100–568 mg of aluminium daily The daily elimination of aluminium in the urine did not exceed 1 mg in any of the patients (6) Recker et al (19) found elevated concentrations of aluminium in the plasma and bones of a patient having ingested aluminium for several years as treatment of a peptic ulcer

In five children who had not been dialysed, Baluarte et al (4) observed symptoms similar to DES The children had ingested large quantities of aluminium but the concentrations of aluminium in the brain were not measured He recommended lowering the dose of aluminium containing compounds to 50–100 mg/kg/day As our patient ingested 43 mg aluminium per kg per day and yet developed the dialysis encephalopathy syndrome even this dosage appears to be too high

Mayor et al (13) showed that the parathyroid hormone increases the gastrointestinal absorption and the deposition of aluminium in the brains of rats Ball et al (3) reported that parathyroidectomy reduced the symptoms of DES The children reported by Baluarte et al (4) had very high levels of parathyroid hormone and so had our patient

All findings indicate that his symptoms were caused by the aluminium He had exclusively ingested aluminium hydroxide We find that aluminium containing compounds should be avoided in the treatment of children suffering from renal impairment with hyperparathyro-

idism Alternative methods to control serum phosphorus levels such as low dietary phosphorus content and subtotal parathyroidectomy (3, 4) should be considered

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Submitted March 4 1980
Accepted May 19 1980

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Acta Pædiatr Scand 69 797–801, 1980

CASE REPORT

SEVERE COMBINED IMMUNODEFICIENCY ASSOCIATED WITH HYPERIMMUNOGLOBULINAEMIA E, EOSINOPHILIA AND IMPAIRED NEUTROPHIL CHEMOTAXIS

B BECK B FRIIS C HEILMANN F KARUP PEDERSEN and N H VALERIUS

From the University Clinic of Paediatrics Children s Hospital Fuglebakken the University Clinic of Infectious Diseases and the University Clinic of Paediatrics Department G Rigshospitalet and the Department of Clinical Microbiology Statens Seruminstitutet Copenhagen Denmark

ABSTRACT Beck, B , Friis, B , Heilmann, C , Pedersen, F K and Valerius, N. H (University Clinic of Paediatrics, Children's Hospital, Fuglebakken, University Clinic of Infectious Diseases and University Clinic of Paediatrics, Department G, Rigshospitalet and [illegible] combined immunodeficiency was found to have coexistent hyperimmunoglobulinaemia E, eosinophilia and impaired neutrophil chemotaxis Based on the literature, a deficient regulatory function of the T cell system is proposed as the basic defect leading to the observed impairment of the cellular and humoral immunity and, probably through hyper immunoglobulinaemia E, to defective neutrophil chemotaxis

KEY WORDS Immunodeficiency, hypergammaglobulinaemia, eosinophils, neutrophil chemotaxis

Since Glanzmann & Riniker first described essential lymphocytophtisis' in 1950 (10), many reports concerning similar conditions—now usually termed severe combined immunodeficiency (SCID)—have been published These findings have established that SCID consists of a heterogenous group of disorders In some cases the humoral defect is not total, and even a few cases with high concentrations of IgE have been published (2 22) Recently the combination of decreased neutrophil chemotaxis and SCID has been reported (18) This paper presents a patient with SCID and coexistent hyper immunoglobulinaemia E, eosinophilia and decreased neutrophil chemotaxis

CASE HISTORY

A 6-week-old boy was admitted to hospital because of pneumonia Family history did not include recurrent infections and pregnancy and delivery were normal The birth weight was 3 7 kg The child had coarse facial features short neck large tongue and dry and scaly skin From the fifth day of life a generalized dermatitis was present and persisted in spite of treatment with topical corticosteroids

The patient was treated with ampicillin later supplemented with methicillin gentamycin 5 fluocytosine and systemic corticosteroids Respiratory insufficiency developed and in spite of artificial respiration the patient died at the age of 12 weeks from bilateral pneumonia Shortly before death transfer factor was given after an intracutaneous test dose The first test dose elicited a violent urticarial local reaction with ensuing universal exanthema A second test dose from another batch of transfer factor gave no reaction

Cultures from nose throat, sterile tracheal aspirate, urine and stools repeatedly grew *Candida albicans* Also *Staphylococcus aureus* and *Staphylococcus albus* and *Enterobacter cloacae* were cultured from tracheal aspirates Microscopical examination of material from tracheal aspirates showed accumulation of foam like material but definite cysts of Pneumocystis carinii were not seen in Gomori's methenamine staining technique

Hematologic findings are shown in Fig 1 Progressive lymphopenia occurred during the course of the disease

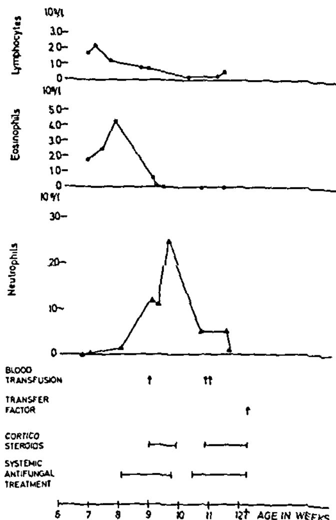


Fig 1 Hematologic findings

Initially, the number of eosinophils was very high but later eosinophils disappeared from the peripheral blood An initial neutropenia was followed by neutrocytosis The bone marrow was hyperplastic with marked eosinophilia but otherwise normal

Concentrations of immunoglobulins in serum are shown in Table 1 The concentration of immunoglobulin E was found to be increased whereas concentrations of immunoglobulin G and immunoglobulin M decreased to subnormal levels during the course of disease Immunoglobulin A remained normal for age and on one occasion was even increased No precipitating antibodies against *Candida albicans* were found in serum

The chest X ray did not show any thymus shadow Skin tests for delayed hypersensitivity were not performed ... Table ... as the ... within number ... normal limits The lymphocytes did not respond with blast transformation on stimulation with either mitogens or microbial antigens The concentrations of the enzymes adenosine deaminase and 5 nucleoside phosphorylase in erythrocytes were normal However the sample was drawn 10 days after a blood transfusion

The polymorphonuclear leucocyte motility, assessed a reversible Boyden chamber as previously described (2 was found severely impaired The chemotactic index w calculated as 1+log ratio between the results of the patie and those of a simultaneously tested healthy contr donor. The chemotactic index towards casein (5 mg p ml) was 0 50 (95% confidence limits 0 70–1 33) ar towards a culture filtrate of *E coli* 0 19 (95% confiden limits 0 67–1 33) In addition the spontaneous migr tion, measured in the absence of chemotactic factor was decreased, with the chemotactic index calculated a above being 0 42 Although severe infections may cau some depression of the chemotactic activity of th leucocytes, a reduction to the level seen in this patie was not observed in a series of patients with severe in fections (N H Valerius unpublished results)

The Nitrobfue-Tetrazolium test was found norma but the child died before a bactericidal test could be per formed

Autopsy revealed enlarged lungs filled with numerous abscesses The thymus weighing 1 8 g, was hypoplastic On microscopical examination only a few lymphocytes and no Hassall s corpuscles were seen The lymphnodes were small few and contained no germinal centers The paracortex was poorly developed and few plasma cells were seen Also the spleen contained few lymphocytes and no germinal centers The other organs were norma *Streptococcus faecalis* was the only organism cultured from the lungs Histological examination revealed foam like material in the alveoli, but *Pneumocystis carinii* could not be identified using Gomori s methenamine staining technique

DISCUSSION

Severe combined immunodeficiency (*SCID*) is a congenital, usually hereditary disease characterized by abnormalities of the T- and B-lymphocyte systems and early onset of severe infections (12, 21) Autosomal recessive, X-linked and sporadic forms have been described (6) Pathological findings include hypoplasia or aplasia of lymphoid tissue and thymus (12) The disorder was previously thought to be caused by deficient stem cell differentiation into T- and B-cells (12) However, recent evidence has suggested that isolated defects in the T-lymphocyte system may also cause failure of B-lymphocytes to differentiate into antibody producing plasma cells (6, 15, 21)

The findings in this child are consistent with *SCID* An early onset of severe infections with several different strains of bacteria, disseminated candida infection and pathological

Table 1 *Immunoglobulins and in vitro lymphocyte studies*

	Age in weeks						
	6	7	8	9	10	11	12
IgE ng/ml (2.4–62.4)[a]			6 900			2 100	
IgA g/l (0.08–0.34)[a]		0.2		0.48		0.26	
IgG g/l (3.11–5.49)[a]		6.0		4.1		2.3	
IgM g/l (0.19–0.41)[a]		0.3		0.13		0.05	
T rosettes							
No		0.142×10^{9}/l					
% (52–80)		18					
B rosettes							
No		0.048×10^{9}/l					
% (8–26)		6					
Immunofluorescent cells							
No		0.103×10^{9}/l					
% (8–29)		13					
PHA/PWM[b] stimulation		No response					
Bacterial antigen[c] stimulation		No response					

[a] Reference interval for age
[b] Phytohaemagglutinin/Poke Weed Mitogen
[c] PPD *Candida albicans* *Staph aureus* *Escherichia coli*

findings resembling those seen in *Pneumocystis carinii* infection (13) were present. Candida precipitins were absent, and levels of immunoglobulin G and immunoglobulin M were low. In vitro studies of lymphocytes showed decreased numbers of T- and B-rosettes in peripheral blood and the lymphocytes did not respond to stimulation with mitogens and antigens. However, the number of immunoglobulin bearing lymphocytes in peripheral blood was within normal limits. Autopsy showed thymic aplasia, lymphnodes with poorly developed paracortical areas and without germinal centers, and few plasma cells were present in lymphoid tissue.

Autopsy findings similar to those seen in this case have been reported in a child with SCID who had a normal number of B lymphocytes in peripheral blood (21). In that child addition of normal T lymphocytes corrected the defective production of immunoglobulins in vitro, thus suggesting a defect T-cell helper function. The failing production of immunoglobulin G and immunoglobulin M in our case combined with the normal number of immunoglobulin bearing cells might be similarly

The combination of SCID, anaphylaxis, hyper-immunoglobulinaemia E, eosinophilia and decreased chemotactic response of neutrophils as seen in this patient, appears very unusual. Hyper-immunoglobulinaemia E has been found in various congenital as well as acquired immune deficiency disorders affecting the T-lymphocyte system such as Wiskott-Aldrich's syndrome, "Buckley's syndrome" and Hodgkin's disease (3, 14, 24). Similar findings have also been recorded in a few patients with SCID-like conditions (2, 22). Tada et al have shown in animal studies that the thymus-dependent immune system is necessary for initiation as well as termination of immunoglobulin E production (22). Therefore hyper-immunoglobulinaemia E in patients with deficient cellular immunity may be due to a deficient regulatory effect of the T-cell system (17). In this context it is of interest that patients with atopic dermatitis and hyper-immunoglobulinaemia E seem to have a slightly decreased T-cell function, and that the degree of hyper-immunoglobulinaemia E is related to the degree of depression of the T-cell function (20). Concerning eosinophilia, Kay et al. have suggested that immunoglobulin

E-mediated anaphylactic reactions release factors chemotactic for eosinophilia (16)

Many reports have established an association between impaired neutrophil locomotion and increased levels of serum immunoglobulin E in patients with dermatitis and undue suspectibility to bacterial infections, most frequently caused by staphylococci (5, 7, 11) The basis for this association remains unclear, but the release of histamin by immunoglobulin E dependent mechanisms may suppress the locomotory response of neutrophils (4) Most patients with this syndrome have had normal cellular immunity Although a few patients have shown defects in cellular immunity in addition to their defective neutrophil chemotaxis (4, 16), none, however, have demonstrated defects as severe as in our patient

The serum concentration of IgA was found slightly elevated on one occasion (Table 1) Some evidence exists that IgA suppresses neutrophil chemotaxis (8, 9), thus suggesting that this increased level of IgA may have contributed to the observed inhibition of neutrophil motility In this respect the present case bears resemblance to an apparently genetic disorder described in four siblings who suffered from repeated infections in association with hyper-immunoglobulinaemia A defective neutrophil chemotaxis and impaired cell mediated immunity However the clinical course in the patient described in this study was much more severe

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Submitted Dec 10, 1979
Accepted April 23, 1980

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E-mediated anaphylactic reactions release factors chemotactic for eosinophilia (16)

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Acta Pædiatr Scand 69 803–806, 1980

CASE REPORT

RING CHROMOSOME 14 IN A MENTALLY RETARDED GIRL

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From the [1]Departments of Clinical Genetics [2]Pediatrics [3]Ophthalmology and [4]Pediatric Radiology Karolinska Hospital Stockholm Sweden

ABSTRACT. Iselius, L., Ritzen, M , Bui, T H , Olsson, K and Eklof, O (Departments of Clinical Genetics, Pediatrics, Ophthalmology and Pediatric Radiology, Karolinska Hospital, *Stockholm Sweden*) Ring chromosome 14 in a mentally retarded girl Acta Paediatr Scand, 69 803, 1980 —A case of ring chromosome 14 in a 12-year old girl, showing mental retardation, epilepsy and minor somatic abnormalities, is described and compared with five previously reported cases with the same chromosome aberration

KEY WORDS Chromosome aberration, ring chromosome 14, mental retardation, epilepsy

Many cases of D ring chromosomes have been reported Most of them have involved chromosome 13 (review in 5) In contrast only five cases of ring chromosome 14 (3 4 6 7, 8) and a few cases of ring chromosome 15 (review in 2) verified by autoradiography or chromosome banding techniques have been reported A few cases of ring chromosome 14 without information about clinical findings are mentioned by Borgaonkar (1) and recently two incompletely reported cases were presented by Schmidt et al (6) and Vekemans et al (8) We wish to report a case of ring chromosome 14 in a 12 year old girl with mental retardation, epilepsy and minor somatic abnormalities

CASE REPORT

Clinical findings

The proposita is the third of five children (one female twin pair) of unrelated parents The mother and father were 25 and 41 years old respectively at the time of birth of the proposita The father who was of Greek origin died three years later of subarachnoid haemorrhage The mother and the other children are healthy No consanguinity cases of malformations or developmental disorders were known in the family

The proposita was born in the 37th week of gestation after an uneventful pregnancy Birth weight 2 870 g length 49 cm The neonatal period was normal except for slight lethargy during the first weeks of life At 8 and 10 months of age she had *two episodes of generalized epileptic* seizures Pneumoencephalography and an angiogram of the right carotid artery showed a frontal abnormality which was interpreted as a small (maximum thickness 5 mm) hygroma *The septum pellucidum* was *wide* as were the parietal sulci Electroencephalogram was unremarkable She was put on anti epileptic medication with satisfactory results She showed evidence of psychomotor retardation at that time which was confirmed by repeated evaluations at 3 and 9 years of age (Fig 1) She started walking at 2 years of age When examined at the age of 10 years her weight was 33 kg and her height was 137 cm (−0 5 S D) She had a general mental retardation especially manifested as poor speech function (total vocabulary about 80 words) Neurological examination was unremarkable except for evidence of mental retardation The following malformations were noted broad flat nose indication of synophrys narrow palpebral fissure high arched palate marked cubitus valgus and increased thickness of the subcutaneous tissue on the dorsal aspects of the hands and feet When last examined at eleven and a half year of age she showed pubertal development (breasts Tanner stage III pubic hair stage III) Routine blood and urine analyses serum electrolytes and tests for aminoaciduria at 7 years of age were unremarkable Her seizures disappeared after age 7

Radiological survey of the skeleton revealed microcrania with marked disproportion between the vault and the facial bones The epiphyses of all long bones were wide and poorly mineralized Cartilaginous exostoses were found medially on both proximal tibiae and on the

fourth metacarpals distally particularly in the proximal



Fig 3 Radiogram of right hand with lateral bowing of radius due to hypoplasia of distal ulna Broad based cartilaginous exostosis of the radial aspect of distal ulna Bizarre configuration of poorly mineralized carpal bones with similar bone structure in the metacarpals and phalanges Slight shortening of metacarpals III–V Cortical thickening of the metacarpals and most phalanges



Fig 4 Right optic fundus showing drusen of macula

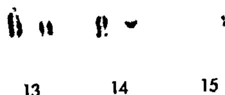


Fig 5 Partial karyotype of the *proposita* showing the D group

palate cubitus valgus short metacarpals hypoplasia of the distal ulnae exostoses) were not reported in the other children with ring chromosome 14 A delineation of a specific phenotype of ring chromosome 14 has to await the description of further cases

ACKNOWLEDGEMENT

This investigation has been supported by grants from the Swedish Medical Research Council (MFR No 3681)

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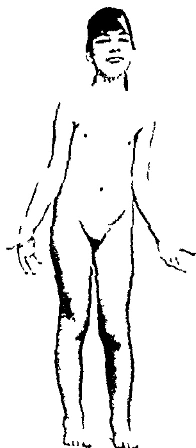


Fig 1 The proband at the age of 9 years



Fig 2 Radiogram of left knee demonstrating wide poorly mineralized epiphysis and proximally on the medial aspect of the tibia a hook like exostosis

phalanges and in most epiphyses of the hand The third to fifth metacarpals were slightly shortened A tendency to thickening of the diaphyseal cortex of the metacarpals basal and middle phalanges was found The skeletal age according to Greulich and Pyle was within normal limits

Eye examination showed moderate hyperopia and astigmatism (+5 00 sph combined with −2 00 cyl ax 180°) Visual acuity at least 20/40 Ophthalmoscopy revealed small hyperemic optic discs with normal disc margins and no prominence The fundic blood vessels were normal Small circular yellow white spots varying somewhat in size and colour were found in the macular region (Fig 4)

Cytogenetical analysis

Chromosome analysis was made on cells from lymphocyte and skin fibroblast cultures from the proposita and on cells from lymphocyte cultures from her mother The chromos
9 years (
analyzed
appeared to be
nal with breakpoints occurring at the telomeric segments of the chromosome (probably at p11 and q3) *An exact* determination of the break points was not possible in the present case The mother had a normal karyotype

COMMENT

The findings in our case, i e psychomotor retardation especially involving speech function and vocabulary epileptic attacks, widened cerebral sulci, and minor malformations such as narrow palpebral fissures, are in good agreement with those made in the earlier mentioned studies Further abnormalities in our patient included high arched palate, cubitus valgus, increased amount of subcutaneous tissue on the dorsal aspects of both hands and feet, hyperopia and abnormal retina The drusen like changes in the retina of our patient are rare at this age and may be connected with the abnormal karyotype However, the child described by Gilgenkrantz et al (3) was reported to have normal eye fundi The minor skeletal abnormalities in

Acta Pædiatr Scand 69 807–808 1980

CASE REPORT

CONGENITAL CHLORIDE DIARRHEA POSSIBILITY FOR PRENATAL DIAGNOSIS

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ABSTRACT Hartikainen Sorri, A L, Tuimala, R and Koivisto, M (Departments of Obstetrics and Gynecology and Pediatrics, University of Oulu, Finland) Congenital chloride diarrhea possibility for prenatal diagnosis Acta Paediatr Scand, 69 807, 1980.—Two pregnancies, which resulted in the births of infants affected by congenital chloride diarrhea (C C D) are presented No method is available for prenatal diagnosis of this disorder In this paper the intrauterine onset of diarrhea is confirmed by amniofoetography and high bilirubin values in amniotic fluid In the second case the amniotic fluid alpha fetoprotein (A F P) was detected to be abnormally high at the 29th gestational week

KEY WORDS Chloride diarrhea, alpha fetoprotein prenatal diagnosis

Congenital chloride diarrhea (C C D) is a genetic disease which is inherited autosomally recessive (8) It is characterized by watery profuse diarrhea with high chloride concentration in the stools This results in newborn dehydration hypoelectrolytemia and abdominal distension within the few first days of life The disease is assumed to have an intrauterine onset Forty five cases have been reported twenty one of them from Finland (3)

The basic defect is an impaired active chloride transport in the distal ileum and colon (4) Without treatment most infants affected by this disorder die neonatally but with adequate electrolyte substitution therapy the infants survive However the long term prognosis is still unknown (3)

Relatively little is known about pregnancies in which the foetus is affected by this disease Pregnancy is usually complicated with polyhydramnion and premature delivery (3) So far there has been no method available for prenatal diagnosis of C C D We now describe two pregnancies which resulted in the births of infants affected by C C D

CASE REPORTS

Case 1 38 year-old 6th gravida who had had four normal pregnancies and births and one early pregnancy abortion Polyhydramnion was noted at the 28th gestational week and despite this no abnormalities in the pregnancy were noted Amniocentesis and anmiofoetography were performed at the 33rd gestational week

There were no external abnormalities of the foetus seen in amniofoetography but the contrast medium (Urografin®) did not concentrate into the intestinal region (X ray films 3 6 and 25 hours after injection) but only traces of it were seen in the distal colon

Amniotic fluid bilirubin was measured spectrophotometrically The optical density (O D) at 450 mμ was high 0 27 (normal O D <0 05 at this gestational age) (6)

Labor started at the 33rd gestational week after the premature rupture of membranes and a girl weighing 1900 g Apgar score 7/8 was born She had no abnormalities but there was abdominal distension profuse watery yellow stools and no meconium Water electrolyte balance was disturbed in the first post natal days of life and hypochloremia was diagnosed The high concentrations of chloride found in the stools are diagnostic of this disorder (4)

Case 2 31 year old second gravida who had had one early pregnancy abortion The uterus was noted to be large in relation to the gestational age at the 17th week of pregnancy Ultrasound examination of the foetus was done at the 26th gestational week The cause of the large uterus was found to be polyhydramnion and no abnormalities in the foetus were seen Amniocentesis was done at the 29th gestational week Bilirubin and alpha

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Submitted March 24 1980
Accepted May 12, 1980

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Acta Pædiatr Scand 69 807–808, 1980

CASE REPORT

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KEY WORDS Chloride diarrhea, alpha fetoprotein, prenatal diagnosis

Congenital chloride diarrhea (C C D) is a genetic disease, which is inherited autosomally recessive (8) It is characterized by watery profuse diarrhea with high chloride concentration in the stools This results in newborn dehydration, hypoelectrolytemia and abdominal distension within the few first days of life The disease is assumed to have an intrauterine onset Forty five cases have been reported twenty one of them from Finland (3)

The basic defect is an impaired active chloride transport in the distal ileum and colon (4) Without treatment most infants affected by this disorder die neonatally but with adequate electrolyte substitution therapy the infants survive However the long term prognosis is still unknown (3)

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Case 2 31 year old second gravida, who had had one early pregnancy abortion The uterus was noted to be large in relation to the gestational age at the 17th week of pregnancy Ultrasound examination of the foetus was done at the 26th gestational week The cause of the large uterus was found to be polyhydramnion and no abnormalities in the foetus were seen Amniocentesis was *done at the 29th gestational week* Bilirubin and alpha

fetoprotein (A.F.P.) measurements were performed. Amniotic fluid bilirubin was abnormally high in this case also (O.D.=0.14). A.F.P. was measured by commercial double blind antibody radioimmunoassay method (manufactured by C.E.A.-I.R.E.-Sorin) and it was abnormally high: 6500 μg/l which is above the 97.6 percentile (medium 500 μg/l) at this gestational age (12). Labor started after the premature rupture of membranes at the 30th gestational week before the planned amniofoetography. A girl weighing 1850 g, Apgar score 3/7, was born. The newborn infant had no other abnormalities but analogical intestinal symptoms as in the first case. The diagnosis was confirmed by measuring water electrolyte balance and electrolyte concentrations of stools at the 3rd day of post natal life.

DISCUSSION

Many facts in these two cases indicate disturbed transport and motility of substances in the intestines, and diarrhea of the foetus in utero.

Visualization of the gastrointestinal canal by amniofoetography was poor, even though the canal was open. The apparent inability to concentrate the contrast medium may be due to accelerated intestinal activity.

In both cases there was a highly elevated bilirubin content in amniotic fluid without signs of Rh immunization. In normal pregnancies small amounts of bilirubin in amniotic fluid are observed, and the amount decreases with advancing gestational age (10).

In the intestine of the foetus there has been proved to be β glucuronidase enzyme activity, which changes bilirubin to the unconjugated form. Unconjugated bilirubin is reabsorbed partly by the foetal enterohepatic circulation and partly by the maternal circulation (7). Because of disturbed resorption and diarrhea high amounts of bilirubin go through the intestine to the amniotic fluid.

In both cases no meconium was seen after delivery of newborns, indicative of prior loss through intrauterine diarrhea.

A.F.P. increases in the amniotic fluid if foetal swallowing or digestion is impaired or absent (1). Principally, A.F.P. passes from the normal foetus into the amniotic fluid by urination (11) and, in addition, traces of it are also produced in the foetal gastrointestinal tract (2). In association with other amniotic fluid proteins A.F.P. is mainly degraded by the process of foetal swallowing and digestion (9) and, thus, the abnormal high value of A.F.P. in our case may be due to impaired digestion and resorption via intestinal mucosa of the foetus suffering from C.C.D. in utero. Because the foetal intestine has been demonstrated to be capable of transferring water, electrolytes and sugar as early as at the 11th gestational week (5), the measurement of amniotic fluid A.F.P. could be useful in prenatal diagnosis of this disorder. Further studies in early pregnancy are needed.

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Submitted Febr. 6, 1980
Accepted May 1, 1980

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Acta Pædiatr Scand 69 809–813, 1980

REVIEW ARTICLE

PRENATAL DIAGNOSIS OF CONGENITAL BLEEDING DISORDERS

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Prenatal diagnosis of many severe congenital disorders is now possible in midtrimester when legal abortion is feasible (1, 2, 3) Amniotic fluid can be aspirated from the 14th week of gestation Chromosomal aberrations and many inheritable metabolic diseases can be diagnosed by examination of cultured fetal amniotic fibroblasts The indications for chromosomal studies include advanced maternal age, familial translocations, a previous child with trisomy, and, in X-linked disorders, fetal sex determination Biochemical studies are indicated when the parents are known carriers of certain inheritable diseases. Disorders may also be revealed by direct analysis of amniotic fluid, e g α fetoprotein when neural tube defects are suspected Ultrasound, which is used to study intra uterine growth, may also disclose gross fetal malformations

Many disorders however, cannot be detected in these ways This is true of most haematological diseases which can be discovered only by examination of fetal blood It was the desire to diagnose the severe haemoglobinopathies that first motivated the search for methods of fetal blood sampling These matters have been reviewed recently (4, 5) Prenatal diagnosis of haemoglobinopathies was possible even when the fetal blood was mixed with maternal blood But diagnosis of other haematological diseases, such as inheritable bleeding disorders, requires fetal blood free from maternal blood

Among congenital bleeding disorders, X-linked haemophilia A and B, and autosomal von Willebrand's disease are preponderant Other congenital deficiencies constitute only a comparatively small group von Willebrand's disease is somewhat more common in Sweden than haemophilia, but clinically severe von Willebrand's disease is much more rare than severe haemophilia About half of the patients with haemophilia A and B have the most severe forms of these diseases The number of registered female carriers of severe haemophilia in Sweden is about 300, but the true number is probably at least twice as high Haemophilia A (factor VIII deficiency) is four times as common as haemophilia B (factor IX deficiency)

Genetic counselling of pregnant women who are known or probable carriers of severe haemophilia has until recently been confined to a recommendation of fetal sex determination Fetal sexing is done from the 14–15th gestational week by amniocentesis and investigation of the karyotype of amniotic cells Many of the carrriers with a male fetus have chosen to terminate their pregnancies to avoid the possibility of bearing a haemophilic son This practice entails a 50% risk of aborting a healthy fetus It has therefore been considered urgent for ethical reasons to develop methods which can diagnose or exclude haemophilia in male fetuses at risk For some years we have known that the antihaemophilic factors and the von Willebrand factor occur in 16–20-week fetuses in small but measurable amounts (6, 7) Prenatal diagnosis of haemophilia and also of von Willebrand's disease

Table 1. *Reference values for VIII CAg (antihaemophilic factor A antigen) and VIIIR Ag (von Willebrand factor antigen) in non-haemophilic fetal samples (no. 8) obtained by fetoscopy at 17–20 weeks of gestation*

1 U of factor VIII is the amount present in 1 ml of pooled normal adult plasma

	VIII CAg (U/ml)	VIIIR Ag (U/ml)	Ratio VIII CAg/ VIIIR Ag	EVF
Fetoscopy sample	0.025–0.20	0.09–0.69		4–30%
Value corrected to a fetal EVF of 37%	0.19–0.35 (M 0.28)	0.62–1.83 (M 1.01)	0.19–0.43 (M 0.30)	

therefore seemed feasible. Refined techniques for obtaining fetal blood as well as the development of sensitive immunoradiometric assays of the antihaemophilic factors have now made it possible to diagnose these diseases before birth.

Fetal blood can be obtained at fetoscopy from the 17th–18th week of gestation (8, 9, 10, 11). The placenta and fetus are located by ultrasound scanning. A fetoscope (Dyonics "Needlescope") is then inserted into the amniotic cavity either transabdominally or transvaginally (12). Under direct vision a vessel on the chorionic plate is selected, and the vessel, which is fetal, is punctured or cannulated and blood is withdrawn. To ascertain immediately whether the blood samples obtained really are fetal, the erythrocyte volume can be measured in a Coulter Channelyzer. In the second trimester, fetal red cells are 40–50% larger than maternal red cells. The fetal origin of the blood sample is then further checked with the Kleihauer-Betke acid elution technique (13). Complications of fetoscopy and blood sampling include fetal bleeding, leakage of amniotic fluid, amnionitis and premature birth. The fetal loss rate, which was originally close to 15%, has decreased with the increasing experience with the technique (4, 14).

Fetal blood samples for coagulation studies are anticoagulated with citrate solution. They are often more or less mixed with amniotic fluid during sampling. The dilution factor can be calculated from the EVF of the mixed sample and the assumption that in the 20th week fetal EVF is 37% (6, 15). An admixture of amniotic fluid to the fetal samples is a disadvantage, since amniotic fluid often contains thromboplastic substances. Such substances may interfere with conventional coagulant assays with false values as a result. It may also be difficult to add an exact amount of citrate to the fetoscopic samples, which will also make coagulant assays less reliable. The difficulties can be overcome by the use of immunological methods for determining factor VIII and factor IX. The antihaemophilic factor A (=factor VIII C, C=coagulant activity)[1] is associated with an antigen (f VIII CAg), which can be assayed with recently developed immunoradiometric methods (16, 17, 18). In severe haemophilia A, f VIII CAg is not detectable in plasma (9). Immunoradiometric methods have been successfully applied to the prenatal diagnosis of haemophilia A (10, 19). Other workers (20) have apparently been successful also with the use of a modified coagulation assay for the same purpose.

In our experience coagulant assays of fetoscopic samples are not reliable enough by themselves but must be supplemented with

[1] Nomenclature of factor VIII complex:
VIII C=antihaemophilic factor A, measured as coagulant activity.
VIII CAg=antihaemophilic factor A, measured immunologically as antigen.
VIIIR Ag=factor VIII related antigen=von Willebrand factor antigen.

immunological assays We have hitherto examined prenatally 5 fetuses at risk for haemophilia A, and our decision was based on the results of the VIII CAg assay This assay has a sensitivity of 0 8% of the normal adult plasma level (=0 008 U/ml) (16) It is not influenced by admixture of amniotic fluid, which does not in itself contain any factor VIII Our reference values for f VIII CAg in 16–20-week fetuses are given in Table 1 They were determined in samples obtained at fetoscopy of fetuses which were then aborted for therapeutic reasons The samples were more or less diluted with amniotic fluid and citrate solution, and the values for undiluted plasma were therefore calculated assuming a fetal EVF of 37% (Table 1) The von Willebrand factor (factor VIII related protein or antigen=f VIIIR Ag) is always determined simultaneously as an internal control Factor VIIIR Ag is a plasma protein which is thought to form a dissociable complex with f VIII C The ratio f VIII CAg/f VIIIR Ag is a valuable aid in the diagnosis (Table 1)

Of the 5 fetuses at risk for haemophilia A, 2 had no detectable f VIII CAg and were aborted Examination of the postabortal fetal plasma confirmed the diagnosis of haemophilia A in these 2 fetuses The other 3 fetuses had detectable f VIII CAg and a ratio f VIII CAg/f VIIIR Ag within the control range The pregnancies with these 3 fetuses were continued and examination of the 3 boys after birth showed them not to be affected

Haemophilia A occurs in several forms According to the level of f VIII C activity severe moderate and mild forms are distinguished The question of prenatal diagnosis arises in the families with severe and perhaps sometimes also with moderately severe haemophilia A In the latter case however, the immunoradiometric assay can diagnose only one of several genetic variants (21)

Haemophilia B (factor IX deficiency) is only one fourth as common as haemophilia A Factor IX is a vitamin K dependent coagulation factor, which is present in 16–20-week fetuses but in only low concentration (6, 20, 22) Prenatal diagnosis of haemophilia B is associated with the same difficulties as is the diagnosis of haemophilia A Thromboplastin contamination of the fetoscopic samples may lead to false high values of factor IX activity We have developed a sensitive immunoradiometric assay also for factor IX, which has been successfully applied in one case with a fetus at risk for severe haemophilia B (22, 23) However, there are genetic subtypes also of haemophilia B, and perhaps not more than 60% of the cases can be diagnosed with immunological methods Another difficulty is that amniotic fluid contains small amounts of factor IX (22) probably almost entirely of maternal origin This has to be taken into account in the calculation of the fetal plasma level of factor IX from the values of the samples obtained at fetoscopy and contaminated with amniotic fluid

von Willebrand s disease is one of the most common inheritable bleeding disorders The basic defect in this disorder is a deficiency of the von Willebrand factor (measured immunologically as f VIIIR Ag), which is normally formed in endothelial cells and present in plasma Also von Willebrand's disease occurs in several genetic variants (24) Patients with severe symptoms comparable to those in severe haemophilia are relatively few, however The inheritance is considered to be autosomal dominant, but there is good reason to believe that many, if not all, patients with the severe forms of the disease are homozygotes or possibly double heterozygotes Prenatal diagnosis of von Willebrand s disease can be considered in families where one severely afflicted child has already been born or where it is known that both parents are affected, or carriers of a von Willebrand gene If von Willebrand s disease, whether severe or mild, is present in only one of the parents, there will, as a rule, be no indication for prenatal diagnostic procedures F VIIIR Ag is readily detected in normal 16–20-week fetuses (6) and one case of prenatal exclusion of von Wille-

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Submitted April 11 1980
Accepted June 2 1980

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brand's disease has hitherto been reported (25)

Other inheritable defects of the coagulation system are rare compared with haemophilia and von Willebrand's disease Most of them are inherited as autosomal recessive traits The symptoms of affected individuals are often not so severe as in haemophilia, and the need of prenatal diagnosis thus small Inheritable platelet disorders are more common, but most of them produce only rather mild clinical symptoms Thrombocytopenias should be diagnosable in utero as normal fetal platelet counts have been established (6, 26) It might thus be possible prenatally to detect such a severe disease as Wiskott-Aldrich's syndrome, which is X-linked and comprises thrombocytopenia and severe immunodeficiency Functional tests of fetal platelets in fetal blood samples (27) or morphological criteria might perhaps be capable of revealing qualitative platelet defects, such as severe thrombasthenia and Bernard-Soulier s syndrome However more work is needed in this area

Tests of fetal blood have been successfully used also for prenatal diagnosis of other severe inheritable disorders chronic granulomatous disease (28), α_1-antitrypsin deficiency (29) and Duchenne's muscular dystrophy (30) although the diagnostic procedure for the last-mentioned disorder seems to be less well defined It should be possible to detect several other diseases in utero by application of the appropriate test to fetal blood samples Even though experience with fetal blood sampling has now become rather extensive the procedure carries a greater risk of fetal loss than ordinary amniocentesis If examination of amniotic fluid fibroblasts could replace the examination of fetal blood, it would mean a great advantage Recent remarkable progress has shown that some forms of thalassaemia and sickle cell anaemia can be diagnosed prenatally by analysis of the DNA of amniotic fluid cells (31, 32, 33) This approach might perhaps in a future be possible also in some forms of congenital bleeding disorders

ACKNOWLEDGEMENT

Supported by grants from the Swedish Medical Research Council (B 80-19X 04997 04 A) Svenska livforsäkrings bolags nämnd för medicinsk forskning and Expressens prenatalforskningsnämnd

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Submitted April 11 1980
Accepted June 2 1980

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NEW BOOKS RECEIVED

H W Sutherland & J M Stowers *Carbohydrate metabolism in pregnancy and the newborn* 1978 558 pp illus Springer Verlag Berlin Heidelberg New York 1979 No price given ISBN 3 540-08798 2

P M Strong *The ceremonial order of the clinic Parents doctors and medical bureaucracies* 267 pp Routledge & Kegan Paul London Boston Henley 1979 £8 95 ISBN 0-7100-0379 X

F Falkner N Kretchmer & E Rossi *Advances in vaccination against virus diseases* Vol 11 *In* Monographs in Paediatrics 74 pp illus S Karger AG Basel 1979 US $20 50 ISBN 3 8055 3016-3

B F Trump & R T Jones *Diagnostic electron microscopy* 401 pp illus John Wiley & Sons New York Chichester Brisbane Toronto 1979 No price given ISBN 0-471 89196 7

H J Kaufmann *Technological developments and trends in pediatric radiology* Vol 7 *In* Progress in Pediatric Radiology S Karger Basel Munchen Paris London New York Sydney 1980 300 pp illus US$99 50 ISBN 3 8055 2953 8

B C L Touwen *Examination of the child with minor neurological dysfunction* 2nd Edition *In* Clinic in Developmental Medicine No 71 Heinemann Medical Books Ltd London £27 00

Traditional birth attendants WHO offset publication No 44 1979 102 pp Sw kr 12 ISBN 92 4-170044-0

J C Job *Cryptorchidism* Diagnosis and treatment Vol VI *In* Z Laron (ed) Pediatric and Adolescent Endocrinology 228 pp illus S Karger, Basel Munchen Paris London New York Sydney 1979 US$59 50 ISBN 3 8055 2998 8

E M Ramsey & M W Donner *Placental vasculature and circulation* 101 pp illus Georg Thime Publishers Stuttgart 1980 DM 75 ISBN 3 13 570601 X

J H Hutchison *Practical paediatric problems* 5th edition 677 pp illus Lloyd Luke (Medical books) Ltd London 1980 £12 00 ISBN 0-85324-143-0

W Yule & J Carr (eds) *Behaviour modification for the mentally handicapped* 298 pp Croom Helm Ltd London 1980 £11 95 ISBN 0-85664 841 8

B Cleyton P Jenkins & J Round *Paediatric chemical pathology* 164 pp Blackwell Scientific Publications Oxford 1980 £5 25 ISBN 0-632 00564-5

Y Rideau *Outlines of muscular dystrophy* 154 pp, illus SEREM Edit POITIERS Cedex France 1978 $17

M H Klaus & A A Fanaroff *Care of high risk neonate* 2nd ed 437 pp illus W B Saunders Company Philadelphia London Toronto 1979 £11 50 ISBN 0 7216-5478 9

F E Johnston A F Roche & C Susanne *Human physical growth and maturation* Methodologies and Factors Vol A 30 *In* NATO Life Sciences 364 pp Plenum Publishing Corporation New York and London 1980 $42 50 ISBN 0-360-40420-6

B Wharton (ed) *Topics in perinatal medicine* 176 pp Pitman Medical Great Britain 1980 No price given ISBN 0-272 795747

BOOK REVIEWS

U Stave *Perinatal Physiology* Plenum Medical Book Company, New York and London 1978 851 pp $71 40 ISBN 0306 30999 8

The well known Physiology of the Perinatal Period from 1970 has appeared in a second edition by Uwe Stave The period since the first edition was published has witnessed an accumulation of so much new information in the field of perinatal physiology that a new edition became necessary The second edition is contained within one volume with 840 double spaced pages and 40 chapters

The chapters have been written by a number of selected experts who represent different epochs in the development of perinatal medicine Even in the first edition the standard of the different chapters varied greatly This is even more evident in this new edition

In comparison with other recent treatises on perinatal physiology this book does not specifically emphasize matters of circulation respiration kidney function and gas exchange but covers a much broader area of perinatal interest growth maturation digestion nutrition development of enzymatic function metabolism of several different groups of substances endocrine functions and aspects of the neuromuscular system together with the subjects mentioned above

Much of the merit of this book derives from the reviews on development of the function of the central nervous system which is covered in four chapters and supplemented by separate chapters on the visual system and the auditory response Several other well written chapters deserve mention e g the one on respiratory gas transport characteristics of blood and hemoglobin Other chapters give an incomplete view of the state of knowledge today—apparently because of inadequate updating of the material It is for example unfair to state that the effect of autonomic nervous system function on pulmonary vasculature in the newborn is as complex and unclear as is the effect on systemic vasculature (page 733)

Each chapter forms a separate and complete review which makes the book easy to read but also causes much overlapping Thus although oxygen transport across the placenta is certainly a main issue in perinatology it is nevertheless not justified to describe it in five different chapters

Despite the uneven standard of the chapters of this book it deserves a place as a source of reference in the libraries of pediatric obstetric and physiological departments

Ingemar Kjellmer

M Miskin *Ultrasound in Pediatrics* 337 pp Grune & Stratton New York 1979 No price given

Ultrasound in Pediatrics a textbook written by several authors, covers the main diagnostic ultrasound modalities with emphasis on their use in children The book is intended for the practitioner of general diagnostic ultrasound to residents and fellows in training and for pediatricians and pediatric surgeons The first half (160 pp) deals with sonoencephalography mainly the A mode technique but also emphasizes the use of B mode scans in neonates and small children This part is a comprehensive review of the diagnostic possibilities of sonoencephalography in the hands of an experienced examiner However for those who do not work daily with neurological disorders much of the information is of little value

The second chapter of 46 pages gives a concise review of the essentials of M mode echocardiography

The subsequent chapters (121 pp) deal with abdominal retroperitoneal and gynecological B mode ultrasound The text is well worth reading as it gives a good survey of ultrasound in these regions The pictures presented with black echos on white background are small but generally of good quality

Apart from the surprisingly large portion devoted to sonoencephalography this book is a good introduction to diagnostic ultrasound The basic principles of ultrasound are discussed Emphasis is laid upon the appropriate use of ultrasound in different clinical situations One short coming deserves comment Real time ultrasound in not considered yet it may be of great diagnostic value not only for cardiac examinations but also for examination of the head and regions below the diaphragm and particularly in small children

Jan Hildell

B C L Touwen *Examination of the child with minor neurological dysfunction* 2nd Edition *In* Clinic in Developmental Medicine No 71 Heinemann Medical Books Ltd London £27 00

There are three broad groups of indications on which children must be examined neurologically The first group consists of neurological disease in its initial stage or suspected neurological disease because of a family history Secondly there are children with obvious neurological diseases and thirdly there are children who are referred for a full neurological examination because of complaints about behavioural and/or learning difficulties which have no apparent neurological cause It is especially this last category of children that the book deals with Dr Touwen s first edition like this second one provides the most detailed guide line for neurological assessment of children between three and twelve years of age The results of different tests are recorded in points which are scored in an Examination Proforma or in a Neurological Profile All tests are exactly defined with reference to procedure recording and significance They are accompanied by good and explanatory illustrations

The book is of great importance for neuropaediatricians especially the ones caring for children with minor neurological dysfunctions

Tradit onal birth attendants WHO offset publication No 44 1979 102 pp Sw Cr 12 ISBN 92-4 170044-0

Th... report on traditional birth attendants (TBA) is aimed

... TBAs

... existing organized health system

Each chapter refers to figures summarized in a special section In this part it is possible to find questionnaires and checklists that have actually been tested and used Whenever possible information is given on actual ex perience of using TBA s in various parts of the world In the report a valuable list of references is also included

We can only hope that this report will fulfil its purpose of providing a guideline for the planning realization and evaluation of nationwide programs for training and utiliza tion of TBAs while other available health services are not yet adequate

Margareta Eriksson

F Falkner & J M Tanner (eds) *Neurobiology and nutrition* In Human Growth Vol 3 Plenum Press New York 1980 606 pp illus $37 50 ISBN 0-306 34463 7

... spectively

The understanding of the biological growth process is of fundamental importance for the paediatrician He must be aware of the different factors behind normal and ab normal development from neurological and behavioural points of view In the present third volume the editors have succeeded to bring together authors with a profound knowledge of developmental mechanisms The different chapters are grouped under three headings Neurobiology Nutrition and History of Growth Studies

Neuroembryology and the development of perception the differentiate maturation of the human cerebral cortex and organization and reorganization in the central nervous system are reviews on experimental research These chapters are followed by more clinically oriented ones in cluding ontogenesis of brain bioelectrical activity and early neurological and behavioural development Without any doubt the first nine chapters together form an ex cellent review of the subject

Chapters ten to sixteen are well written and give a com prehensive view of the problem of nutrition and growth in relation to protein intake environmental and genetic factors epidemiological considerations and energy malnutrition ... of obesity is discussed

This excellent volume of Human Growth ends with an ... work ... from the ...

Human Growth volume 3 is invaluable to all those in terested in human development and is recommended to libraries of paediatric clinics

Ovar Eeg Olofsson

Yves M Rideau *Outlines of Muscular Dystrophy* Serem Edit Poitiers Cedex France 1977 160 pp illus $17

This monography was first published in French in 1977

... good description of the various types of muscular dystro phy their genetical background and clinical courses The author has a very thorough knowledge of the muscular dystrophies and he gives a very comprehensive easily read description of them illustrated with excellent figures and tables The schemes for clinical evaluation and follow up of the patients are easily understandable His analysis of scolios development and the derangement of lung func tion during the disease is very instructive

This volume is highly recommended for those who deal with muscular dystrophies It gives a better understanding of this progressive disease and how to inform and treat these patients

Ingrid Bjerre

H J Kaufmann *Technological developments and trends in pediatric radiology* Vol 7 In Progress in pediatric Radiology S Karger Basel Munchen Paris London New York Sydney 1980 300 pp illus $99 50 ISBN 3 8055 2953-8

The first part of this volume concerns CT scanning and echography in infancy and childhood On the basis of data collected in Paris from about 3 500 pediatric CT examina tions of the skull Diebler et coll analyse how much this method contributed to the diagnosis of various neoplastic developmental and inflammatory mass lesions found in 327 of the cases studied The experience gained from this large material confirms the great value of early CT scanning of young patients with suspected space-occupy ing intracranial lesions Supplementary conventional neuroradiologic examinations were required in half of the cases

Brasch & Gooding present their first experiences with extracranial CT in children The findings in the chest abdomen pelvis and retroperitoneum are also exem plified and the radiation doses determined for various scanners are discussed

The pediatric use of abdominal echography is described by Le Quesne who accounts for the findings obtained with the B mode technique Dillon & Meyer survey the methods developed for echocardiography and some of its applications in the investigation of cardiac function and anatomy in infancy

Acta Pædiatr Scand 69: 816–819, 1980

BOOK REVIEWS

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logical dysfunctions

Marcel d'Avignon

Laird G Jackson & R Neil Schimke (eds) *Clinical genetics A source book for physicians* John Wiley & Sons Inc New York 1979 652 pp £21 50 ISBN 0-471-01943-7

The aims of this book are well expressed in the preface The contemporary practicing physician no matter what his area of expertise usually becomes uneasy when the conversational topic turns to inherited disease This feeling is understandable when considered in its perspective because only the most recent medical school graduates have had much experience in the application of genetic principles to man Also geneticists have tended to publish their interesting case material in the genetics journals which are hardly a place where they will be encountered by the ordinary physician Those of us interested in clinical genetics have broadened our literary horizons over the past few years However this has not substantially improved the picture for the non geneticists since the various papers are now scattered throughout a host of both general and special journals Standard genetics texts are usually very basic in their orientation and frequently contain little information of clinical relevance More specialized texts are available but unless a physician has a nearly unlimited budget (and a similar amount of time) he cannot hope to encompass the field

With all the above in mind the editors have compiled most of the current information in medical/clinical genetics in a single volume emphasizing its clinical application The book is divided into two major sections Half of the book describes genetic multisystem disorders including chapters on cytogenetic disorders genetics and cancer genetic disorders of the immune system genetic metabolic disorders heritable connective tissue disorders and pharmacogenetics Half of the book is devoted to organ system diseases including chapters on genetics of cardiovascular diseases genetics of endocrine diseases genetics of the gastrointestinal tract genetics and hematology genetic diseases of skeletal muscle heritable neurologic diseases hereditary hearing loss heritable diseases of the kidney genetic disorders of the respiratory system heritable skeletal dysplasias genetic disorders of the skin and hereditary defects of teeth and oral structures The book starts with a chapter on the basis of inheritance and ends with two chapters on genetic counseling and a glossary of genetics and cytogenetics

All chapters included in this volume are interesting well written and likely to be of direct pertinence to clinical geneticists and those concerned with genetic counseling The book is an important source of information especially since it presents a broad and accurate approach to clinical genetics The editors have undoubtedly succeeded in producing an integrated volume that can serve as a focal source for inquiry into a specific genetic disease in a designated organ system The book is highly recommended for physicians including those with little or no background knowledge of medical genetics for students and for nurses and paramedical personel who come in contact with patients with heritable disease

Felix Mittelman

P Casaer *Postural behaviour in newborn infants* 112 pp , illus William Heinemann Medical Books Ltd London J B Lippincott Co Philadelphia 1980 Sw kr 128 — ISBN 0-4330-5148 5

In professor Prechtl s Department of Developmental Neurology in Groningen Dr Casaer has made a most extensive study of 68 healthy newborn infants Polygraphic and video recordings were made when the babies were in different positions supine and prone and when they were asleep and awake Similar observations were also made when the baby was carried by his nurse or placed in a baby seat or in a rocking table with head holder Postural behaviour in awake newborns changed according to their orientation in space Head lifts occurred frequently in the prone position but rarely in the lateral and never in the supine These observations suggest that posture and movement are already closely linked in the newborn When the baby fell asleep the active postures disappeared the face slid to one side and the legs and arms fell into fully supported positions

The movements are more extensive and last longer when the newborn is in a baby seat than in a horizontal position He makes more movements in the supine than in the prone position In the baby seat the newborn is awake more often and for longer periods which might suggest that the mechanisms controlling his general behaviour are influenced by his posture Clinical implications are that newborn infants might benefit from a nursing schedule in which the position is changed regularly The nursing posture for abnormal infants should be based on an analysis of the individual baby s problems e g hyperexcitable babies might do best in the stable prone position whereas newborns with neck extensor muscle hypertonia would be better placed in a lateral position to avoid too frequent and too vigorous head lifts

The fact that previous posture has an effect on subsequent postural behaviour in a group of normal newborns suggests that the squint baby syndrome and infant scoliosis result from a slight imbalance between a postural preference for the left or the right and are linked to the newborn s capacity to react against gravity

Marcel d'Avignon

A further diagnostic contribution is made by Mikity & Wieler who report their experiences with oblique conventional tomography of the lung hilum in children

The second part of the book contains a special treatment article on therapeutic catheter procedures written by Stannard & Fellows and the comments made by several authors on this article The procedures discussed include atrial septostomy embolisation of the hepatic splenic and renal arteries of broncho pulmonary arterial shunts and of arterio venous malformations of the brain and spinal cord, control of bleeding from esophageal varices and gastro intestinal ulcers closure of patent ductus arteriosus atrial septal defects and aortic pulmonary collaterals and retrieval of cardiovascular foreign bodies

The book offers important knowledge of new radiologic technology and its application to pediatric problems The references are numerous and up to-date

Georg Theander

T M Field *Infants born at risk* Behaviour and Development SP Medical and Scientific Books New York London 1979 498 pp illus £20 25 ISBN 0 89335-057 5

This volume deals with infants born at risk because of perinatal medical complications and the subsequent development of these infants evaluated in many different ways during follow up studies The first two chapters contain an overview of prenatal factors that influence the later outcome and the ability of the neonate to interact with its surroundings Especially the predictive value of neonatal visual perceptual behaviour is important as later intellectual functioning may be correlated to neonatal visual functioning Brainstem electric response audiometry has proved to give adequate information of the hearing capacity of normal babies and can also demonstrate significant hearing impairment in high risk infants In combination with the visual perception test an even better prognostic value can possibly be obtained

Of special interest are the chapters on prematurity with or without accompanying illness In a carefully controlled study Caputo et al report that prematurely born children later in middle childhood show cognitive deficits mainly owing to visually mediated malfunction No correlation was found for verbal or auditory components These deficits were based on a minor central dysfunction rather than on a peripheral lesion and were possibly associated with a limitation of brain development

In a series of studies preterm infants with and without RDS but matched for gestational age and born during two different time periods (1972–1973 and 1975) were compared The infants born during the later period performed better on the Bayley scales of Infant Development but both groups showed a delay in hearing and language development The importance of early intervention and stimulatory programs especially for sensimotor and communication development is discussed

The studies on early neonatal behaviour of full term as well as pre term infants include many new data which may be used prognostically to evaluate infants subsequent interaction with their parents The results of these studies did not show that separation alone would fully account for parenting disorders Even when v premature and sick infants were separated for l periods many parents felt very close to them and w able to care for them in a normal adaptive way T maternal affective state and the role and support by t father seem to be components that are equally import to overcome potential dangers in early separation of newborn infant from the mother

Supplemental sensory stimulation of premature infa is still a controversial issue The type contingency du tion and timing of the stimulation as well as the pers who administers it are factors of importance So far can assume that individual sensory stimulation is an i portant part of the care for infants at risk Fut research will have to clarify what kind of stimulation most appropriate in the individual case Methodolog problems are discussed in the last chapter Repea assessments successive observations and attempts distinguish between group findings and individual ferences seem to be of great value

It is a pleasure to recommend this well written volu to all of us who are working with the delicate task to c for infants born at risk and their families

Peter de Chateau

Viral respiratory diseases Report of a WHO Scienti Group *World Health Organization Technical Re Series* No 642 1980 63 pp Sw fr 4 – ISBN 92- 120642 X

Communicable diseases of the respiratory tract ar major cause of morbidity and mortality all over the wor and result in large economic losses For this reason WH has decided to expand its activities to include the contr of these diseases The present report was produced point out the role played by viral infections with spec reference to underprivileged populations

The report claims to contain the latest available stati tics on the mortality from acute and chronic respirator diseases These diseases are common causes of deat at the two extremes of age It further gives a summar of the common etiological agents supplemented wit figures from the WHO virus reporting system In add tion a short chapter is included on laboratory investiga tion of viral respiratory disease However the statemen that the demonstration of a viral etiology may preven unnecessary administration of antibiotics is questionable since a concomitant or superimposed bacterial infectio can seldom be ruled out

Surveillance programs are discussed and it is suggested that surveillance units should make use of existing WHO national influenza laboratories Research needs are also discussed The report contains recommendations on the management of respiratory disease and information on the development of antiviral drugs and the role of immunization In an appendix there is a guide for training health workers at a community level

I regard this report as an information about the work WHO is carrying out within the field of viral respiratory diseases Most of the facts given are also available in standard text books

Margaret Eriksson

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Marcel d Avignon

Acta Pædiatr Scand 69 820, 1980

ANNOUNCEMENT

INTERNATIONAL SYMPOSIUM ON THE INTENSIVE CARE OF CHILDREN

The symposium will be held in Ljubljana the capital of Slovenia Yugoslavia from September 23rd to 25th 1981 The symposium will take place in the new Festival Hall, 'Ivan Cankar' For further information write Prim Dr Pavle Kornhauser, M D, Pediatric Surgical Department University Medical Centre, 61105 Ljubljana, Zaloska 7, Yugoslavia

Preparation of Manuscripts

Contributors are requested to pay particular attention to the following rules governing the preparation of manuscripts *Failure to follow these rules is a frequent cause of delay in the publication of articles and may result in rejection of otherwise acceptable manuscripts*

Manuscripts should be sent, in duplicate, to the office of the Managing Editor, adress Acta Paediatrica Scandinavica, Professor C. G Bergstrand, Department of Paediatrics, S t Gorans Sjukhus, S-112 81 Stockholm, Sweden mailed flat and transmitted by first class mail They should be double-spaced, typewritten on one side of the paper with wide margins Authors should exercise *particular care in the preparation, notation and description of figures* and tables Each table must be ac- [illegible]

A separate *title page* is necessary and should bear a) the title, b) the initials and names of the authors, c) the institution of origin Below the list of references the principal author should state his address to which the proofs are to be sent

A short *abstract* not exceeding 200 words must accompany each manuscript informing about problem, methods, results and conclusions Unexplained abbreviations and references are not allowed The abstract must be typed on a separate sheet and styled as illustrated

ABSTRACT Köhler, L. and Holst, K. (Department of Paediatrics, University Hospital, Lund, Sweden) Dental health of four-year-old children. Acta Paediatr Scand 00
An unselected population of 1 567 four year-old children
KEY WORDS Pre-school children caries, gingivitis

A *summary* is usually not necessary but may be allowed if the author (or the Editor) finds it essential A summary should be typed *immediately after* "discussion"

References to the literature should be limited to those quoted by the author References should be numbered *consecutively* in the order in which they are first mentioned in the text Identify references in text, tables and legends by arabic numerals (in parenthesis) Abbreviate *in accordance with the style of Index Medicus*

Correct forms of references

Standard Journal Article

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The symposium will be held in Ljubljana the capital of Slovenia, Yugoslavia from September 23rd to 25th 1981 The symposium will take place in the new Festival Hall, Ivan Cankar' For further information write Prim Dr Pavle Kornhauser, M D , Pediatric Surgical Department University Medical Centre, 61105 Ljubljana, Zaloska 7 Yugoslavia

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An unselected population of 1 367 four year-old children
KEY WORDS Pre-school children, caries, gingivitis

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